Neurodegeneration

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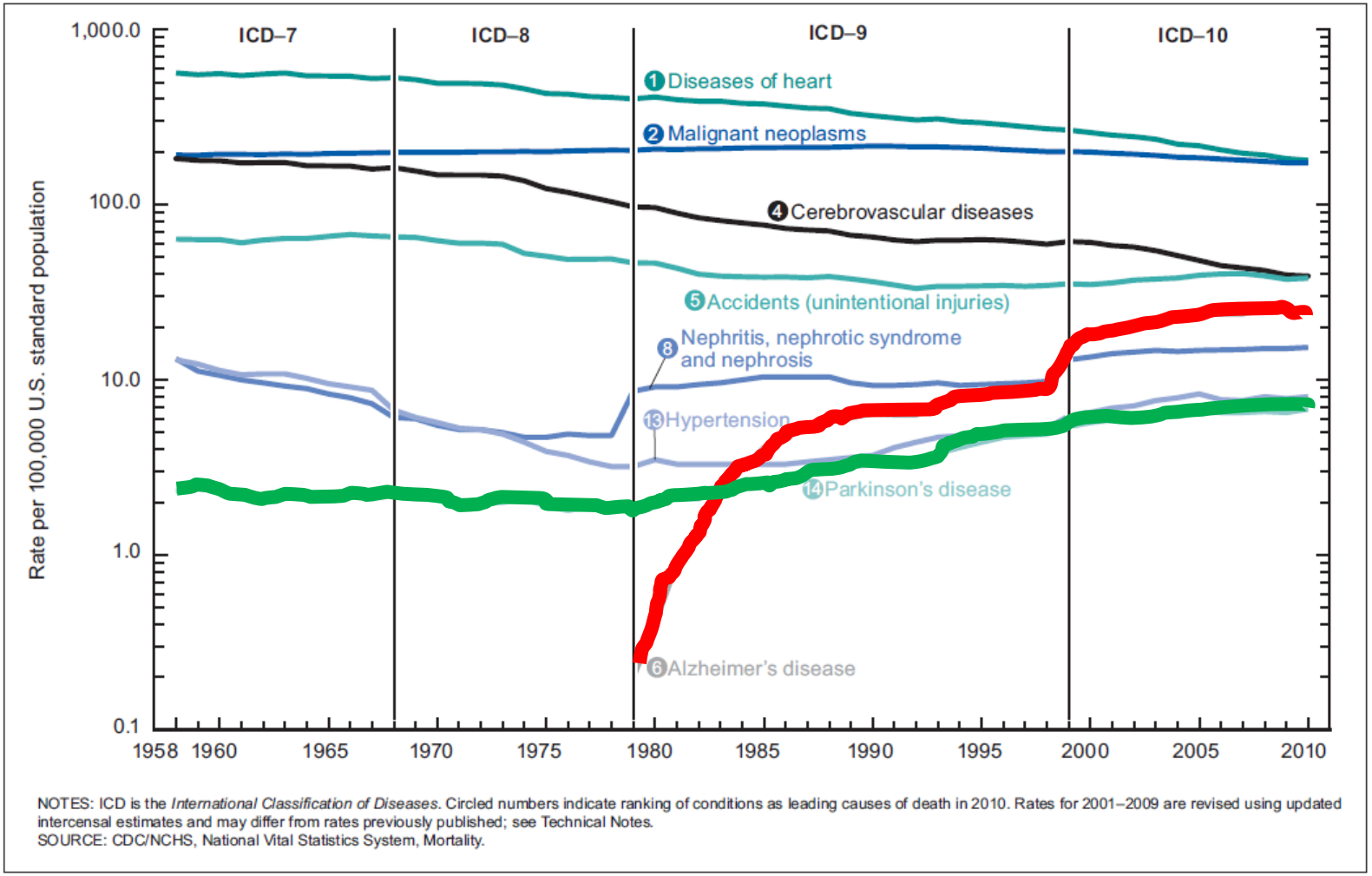
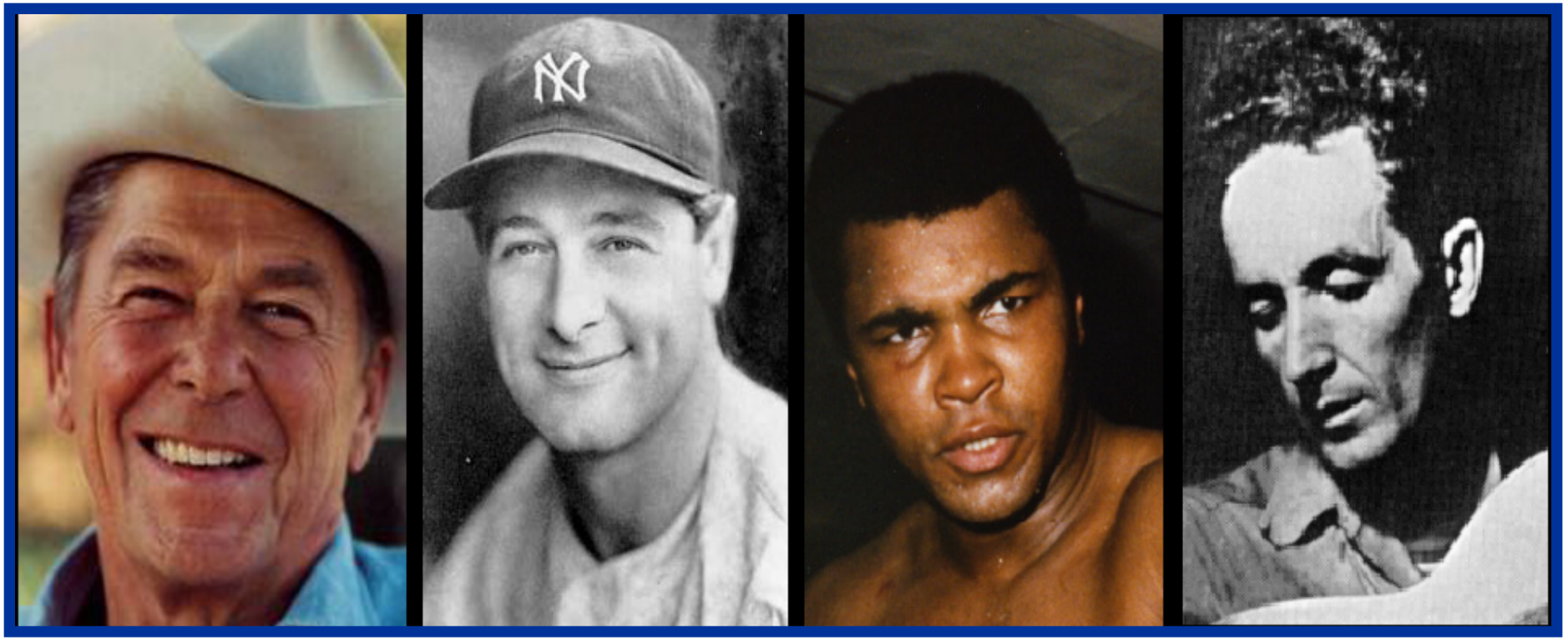
Leading causes of death in the United States 1958-2010



NOTES: ICD is the *International Classification of Diseases*. Circled numbers indicate ranking of conditions as leading causes of death in 2010. Rates for 2001–2009 are revised using updated intercensal estimates and may differ from rates previously published; see Technical Notes.
SOURCE: CDC/NCHS, National Vital Statistics System, Mortality.

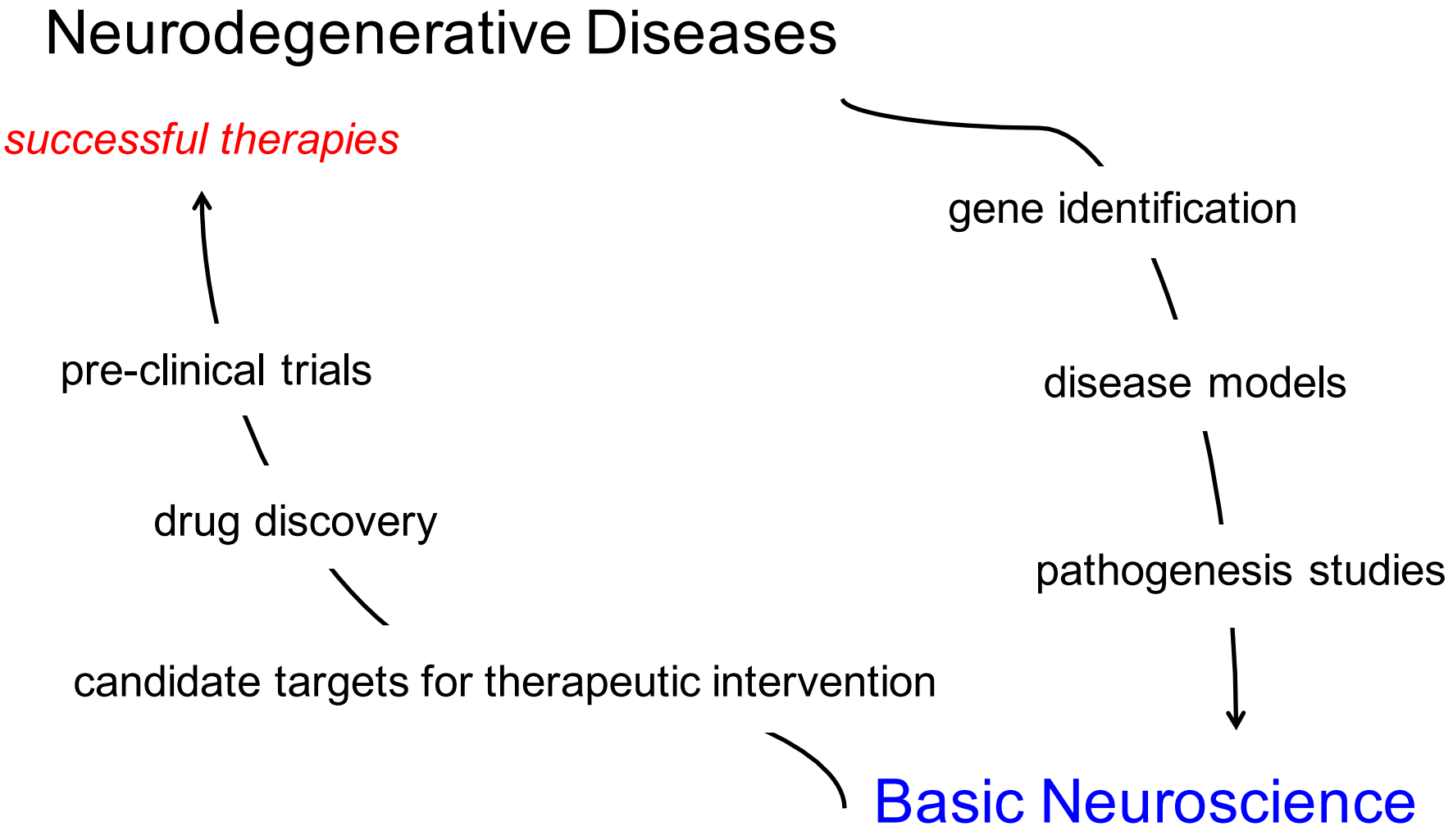
- It is estimated that up to 14 million Americans (>100 million worldwide) will suffer from Alzheimer's disease by 2050.

Age-related neurodegenerative diseases

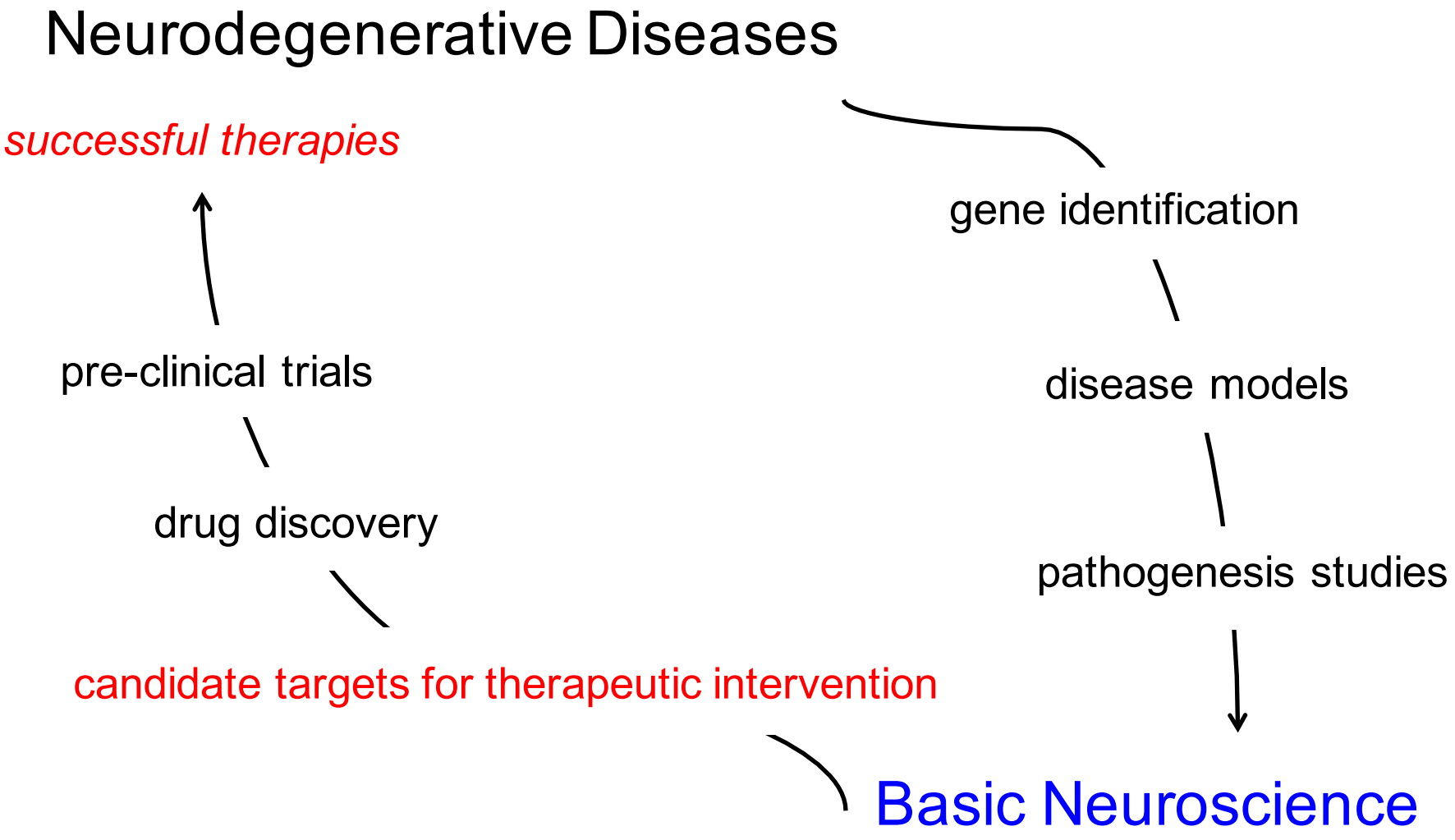


Reagan	Gehrig	Ali	Guthrie
Alzheimer Disease	Amyotropic Lateral Sclerosis	Parkinson Disease	Huntington Disease

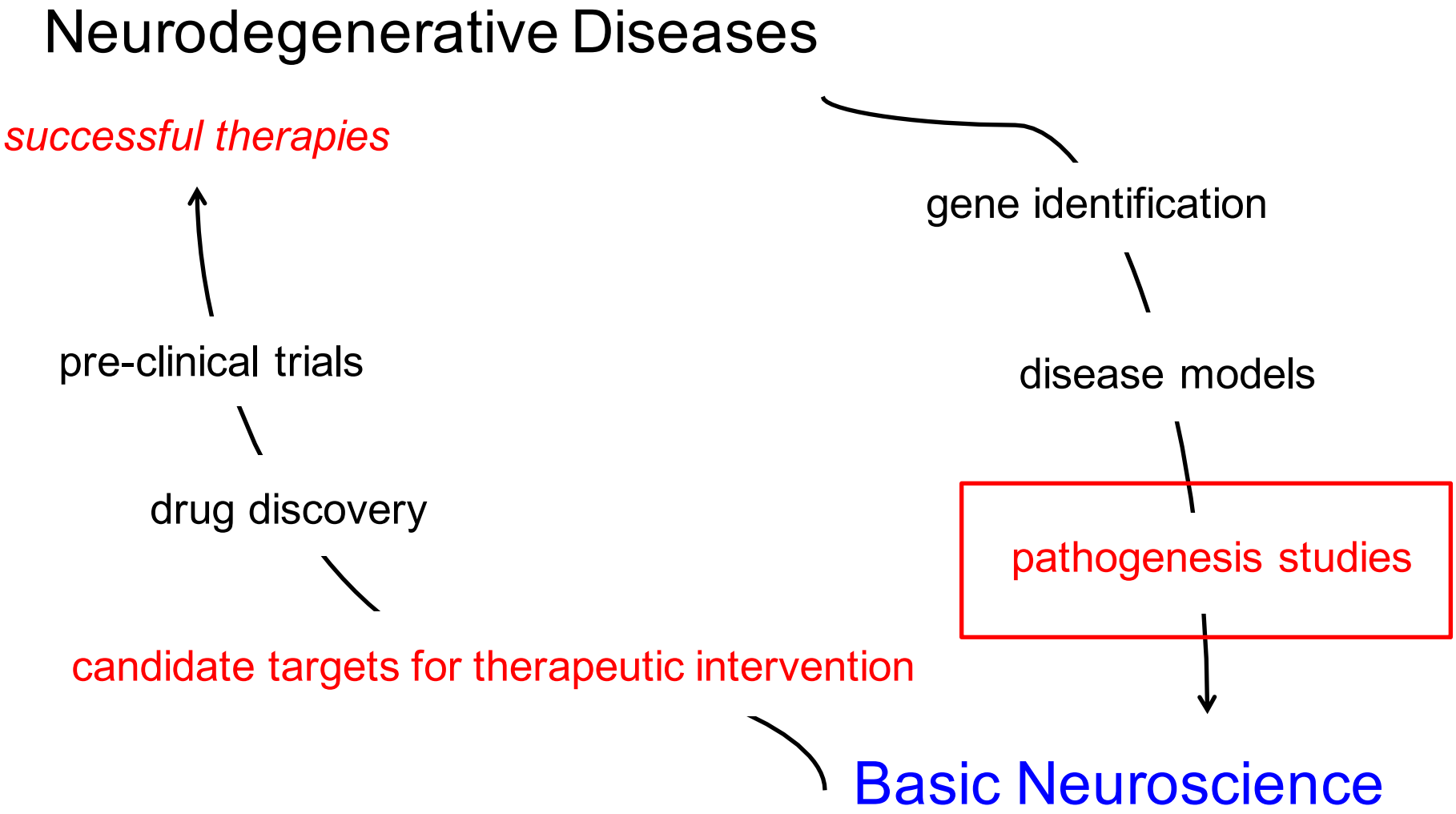
Translational Neuroscience



Translational Neuroscience



Translational Neuroscience

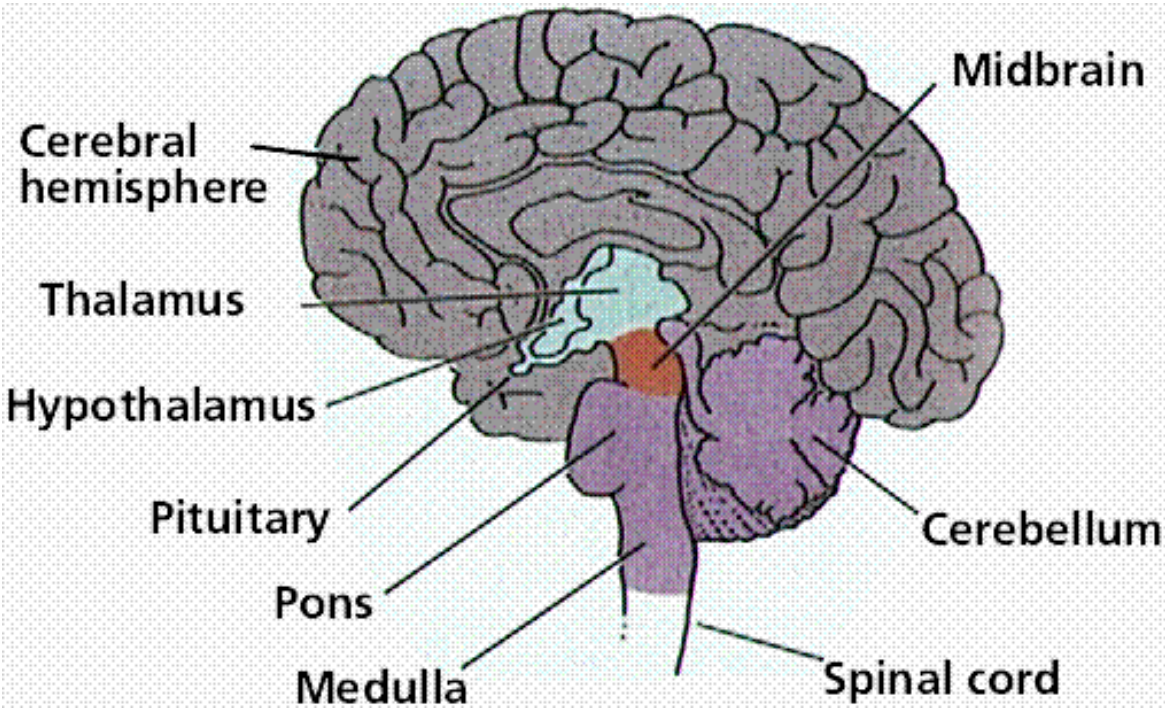


Examples of neurodegenerative diseases

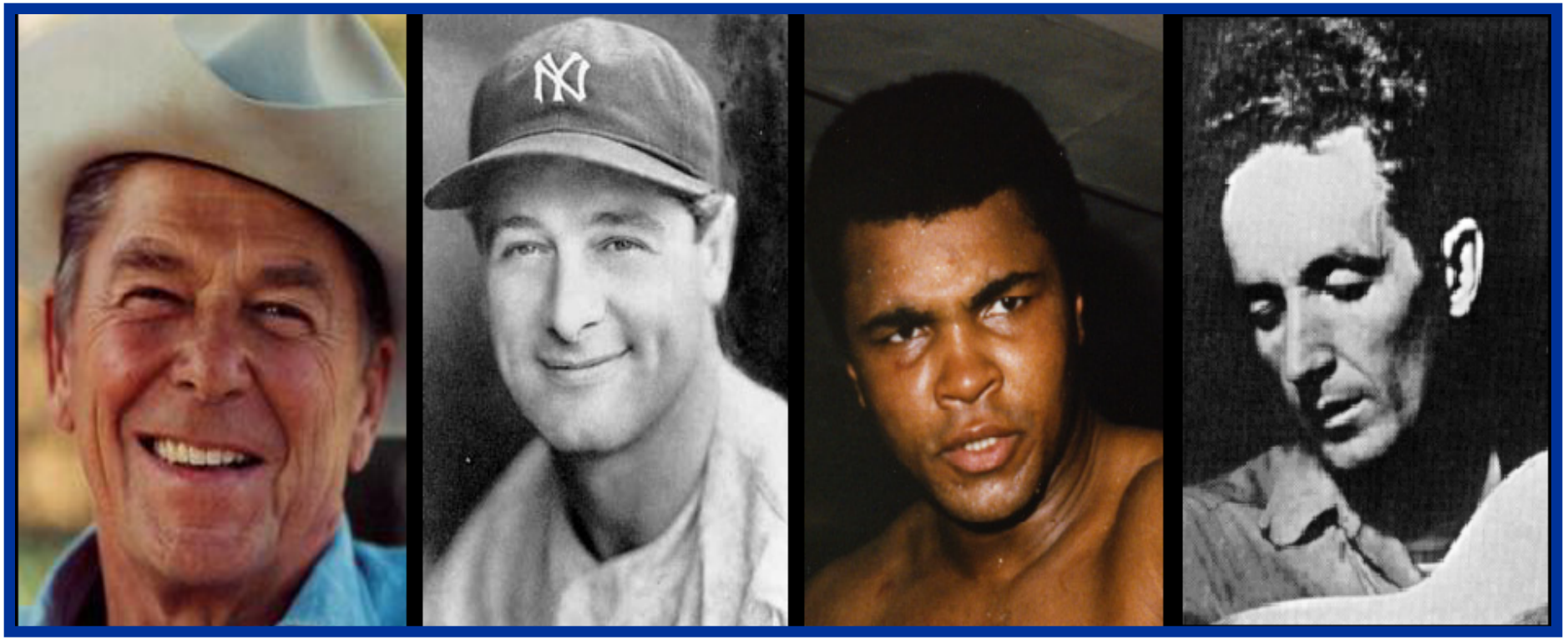
- Alzheimer's disease
- Parkinson's disease
- Tauopathies
- Frontotemporal lobe degeneration / dementia
- Amyotrophic lateral sclerosis
- Polyglutamine disease
- ataxia
- Retinal degenerative disease
- Multiple sclerosis
- etc.

Age-related neurodegenerative diseases

	primary target neurons
Alzheimer's disease	cortical and hippocampal neurons
Parkinson's disease	dopaminergic neurons
Huntington's disease	striatal neurons
Spinocerebellar ataxia	Purkinje cells
Prion disease	cortical neurons



Common themes link most neurodegenerative diseases



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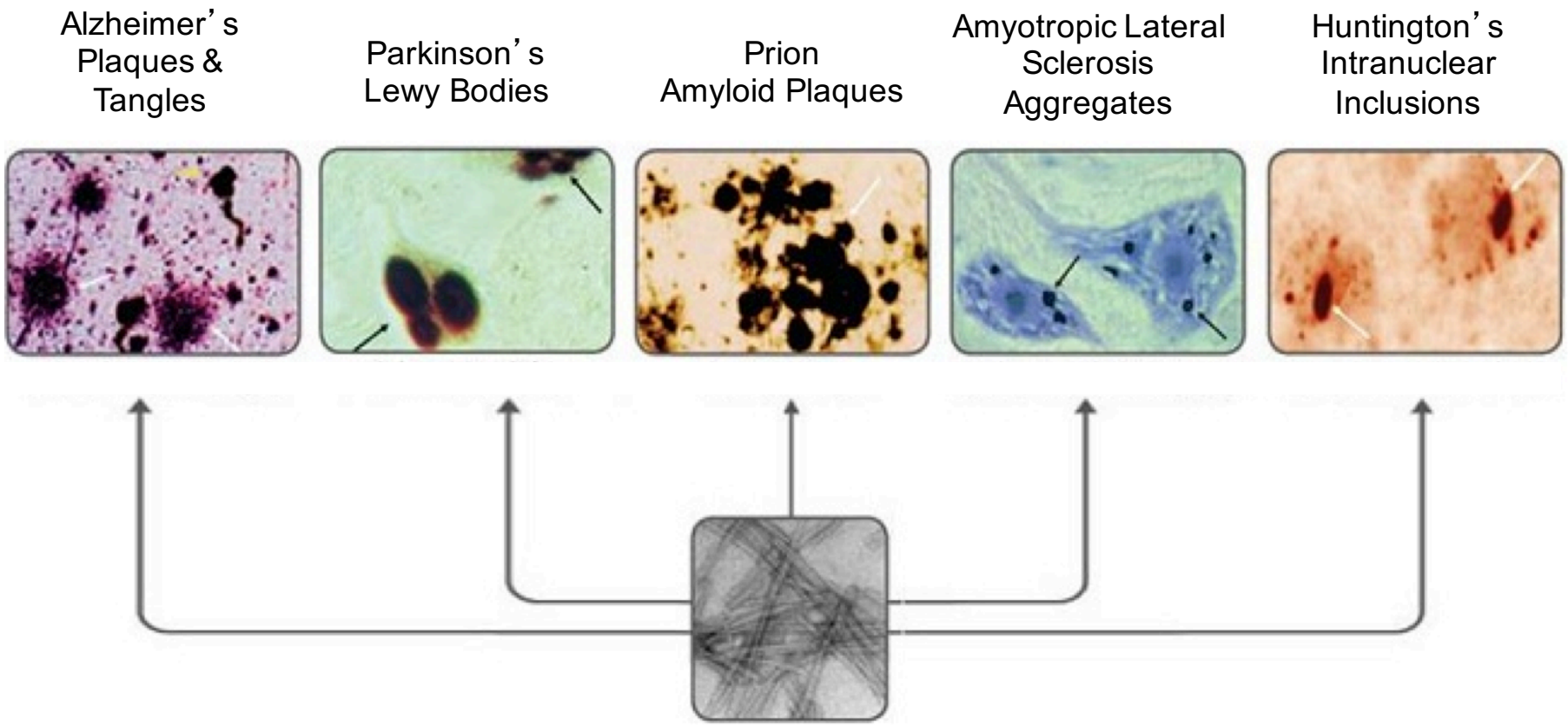
Neurodegenerative diseases share some disease features: e.g. protein misfolding/aggregation



Neurons interact with neighboring glial cells

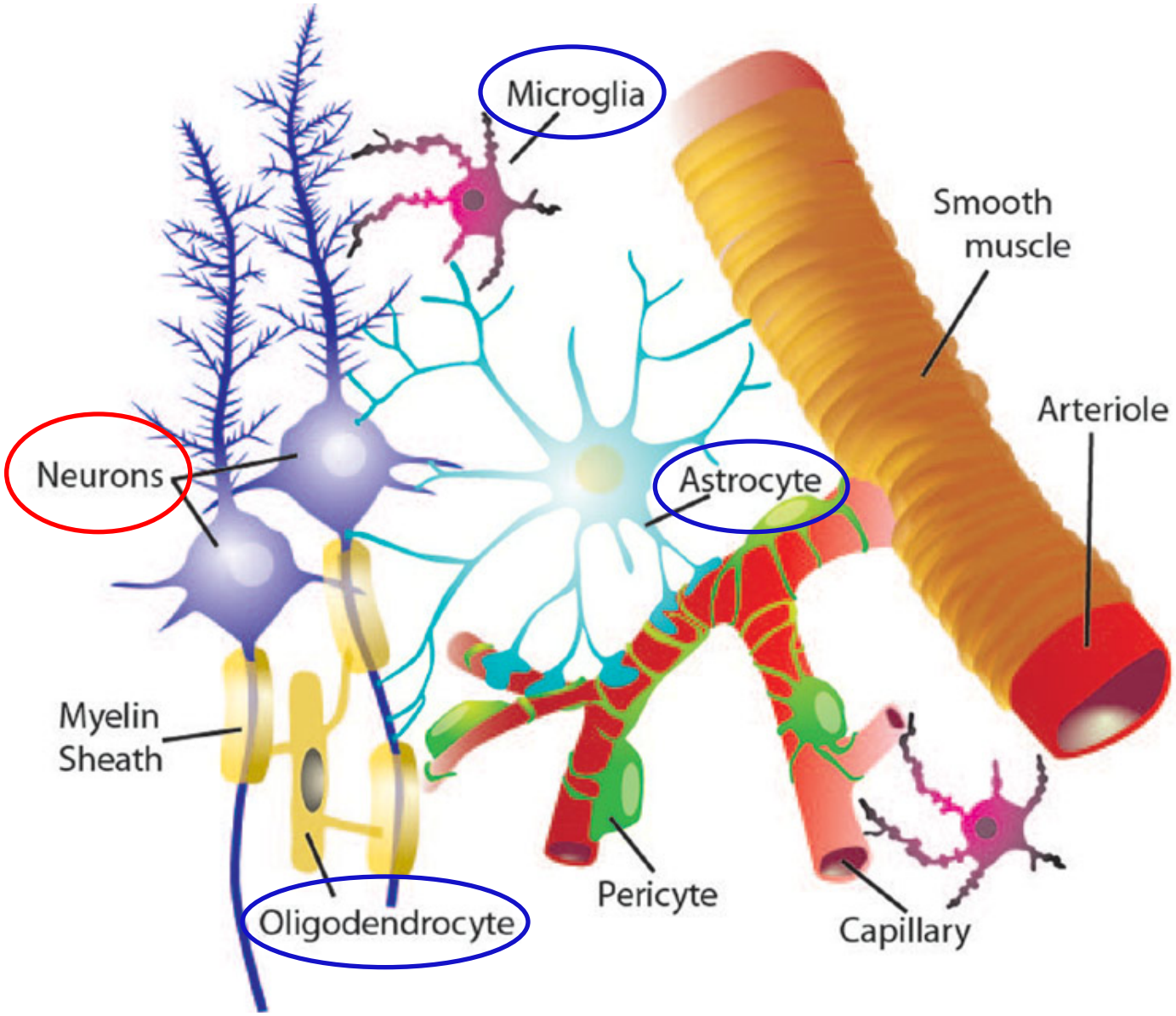


Image courtesy by Dr. Grutzendler

Types and Functions of Glia in the CNS (or PNS)

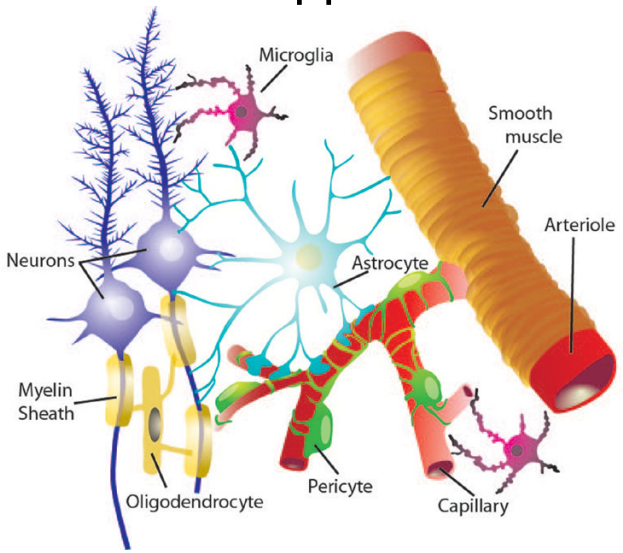
- Oligodendrocyte (or Schwann cell)

  - provides the insulation (myelin) to neurons.

- Astrocyte

  - Star-shaped cells that provide physical and nutritional support for neurons:

    - regulate content of extracellular space
    - hold neurons in place
    - transport nutrients to neurons
    - clean up brain "debris"
    - digest parts of dead neurons



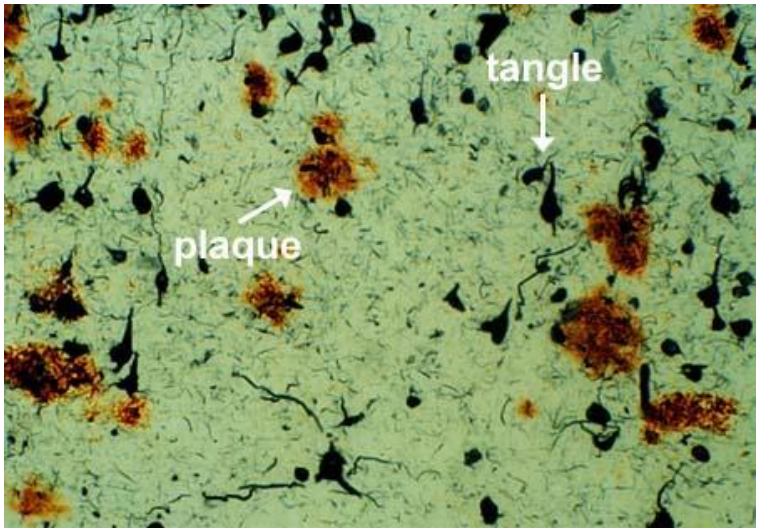
- Microglia

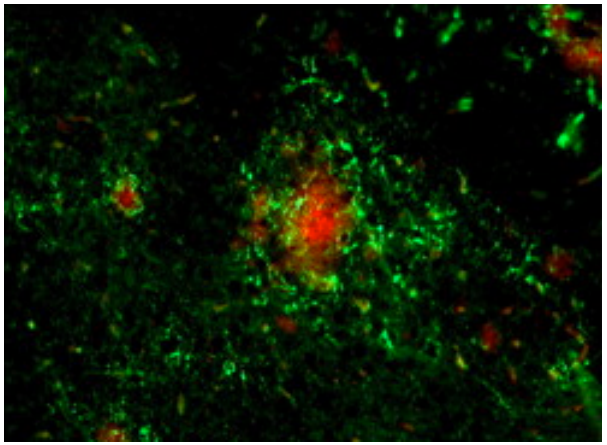
  - The resident macrophages of the brain and spinal cord, thus act as the first and main form of active immune defense.

  - digest parts of dead neurons, like astrocyte.

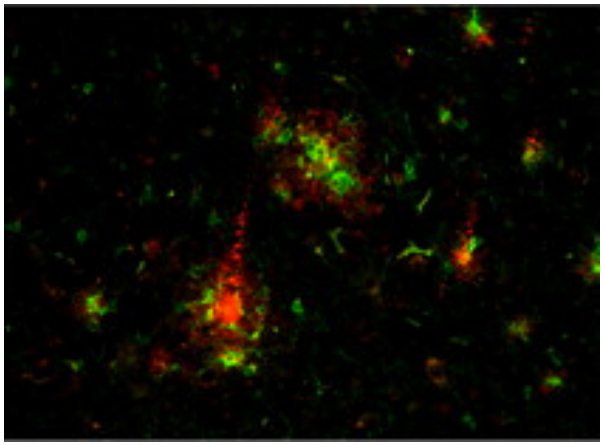
Glial cell proliferation/activation surrounding amyloid plagues : Neuroinflammation in the pathology of Alzheimer's disease

Alzheimer's Plaques & Tangles





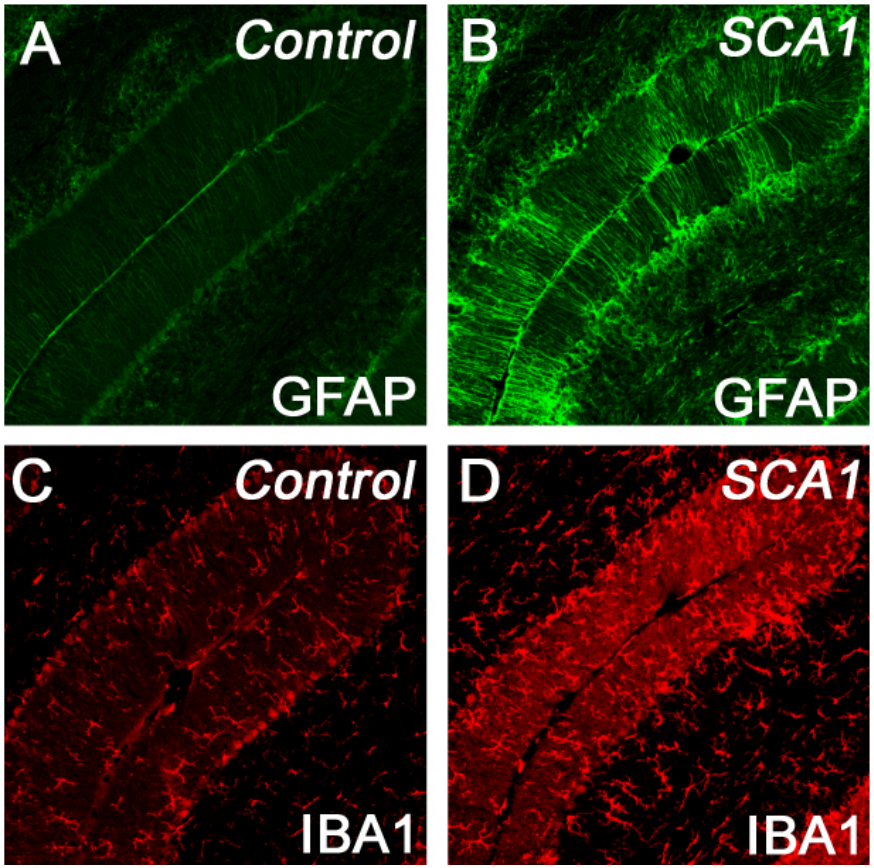
Plaque Astrocyte (GFAP)

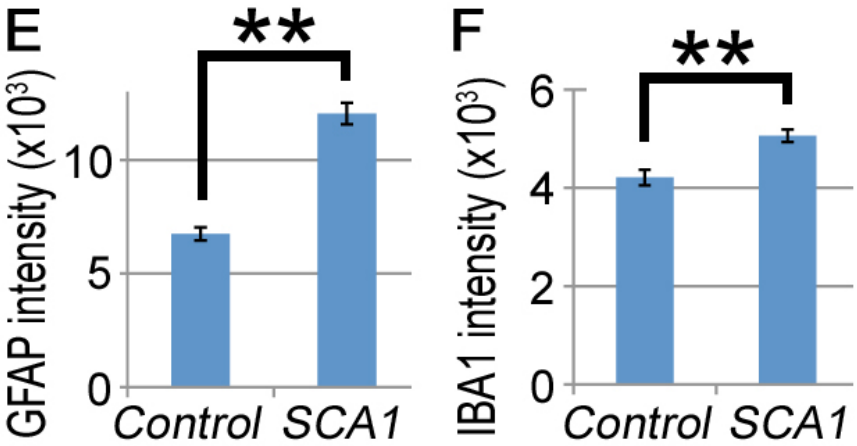


Plaque Microglia (Iba-1)

- Deposition of amyloid-β peptide drives cerebral neuroinflammation.

Glial cell activation in the cerebellum of spinocerebellar ataxia type 1 (SCA1)



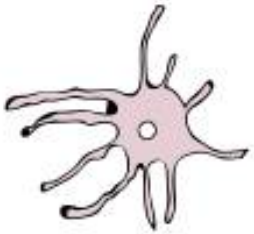


**P<0.001, *t-test*, n=4

GFAP; astrocyte marker

IBA-1; microglia marker

Non-cell autonomous pathogenesis in neurodegenerative diseases

		Involvement of other cell types		
	 *primary target neurons*	 *astrocytes*	 *microglial cells*	 *Schwann cells or oligodendrocytes*
Alzheimer's disease	cortical and hippocampal neurons	not directly tested	microglial dysfunction contributes to pathogenesis[1]	not directly tested
Parkinson's disease	dopaminergic neurons	express enzyme that induces toxicity[2]	their activation precedes neurodegeneration[3]	elevated expression in oligodendrocytes suffices for disease[4]
Huntington's disease	striatal neurons	mutant expression renders neurons vulnerable in culture[5]	their activation occurs early and progresses with disease[6]	not directly tested
Spinocerebellar ataxia	Purkinje cells	mutant expression in Bergmann glia suffices for disease[7]	not directly tested	not directly tested
Prion disease	cortical neurons	PrP^{C} expression suffices for disease[8]	microglial activation decreases prion infection[9]	probably not important for pathogenesis[10]

Ilieva et al., J. Cell Biol. 2009

Lecture Overview

- Alzheimer's disease
- Nucleotide expansion (neurodegenerative) disorders and their key pathogenic mechanisms
- Relatively new disease pathogenesis
  - spreading of mutant disease-causing aggregates

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Alzheimer's Disease (AD)
Clinical Overview

- The most common form of dementia and neurodegenerative disease that causes problems with memory, thinking and behavior.

- About 5 million people in the US currently have the disease and number is expected to increase up to ~14 million by 2050.

- Symptoms usually develop slowly and get worse over time, becoming severe enough to interfere with daily tasks.

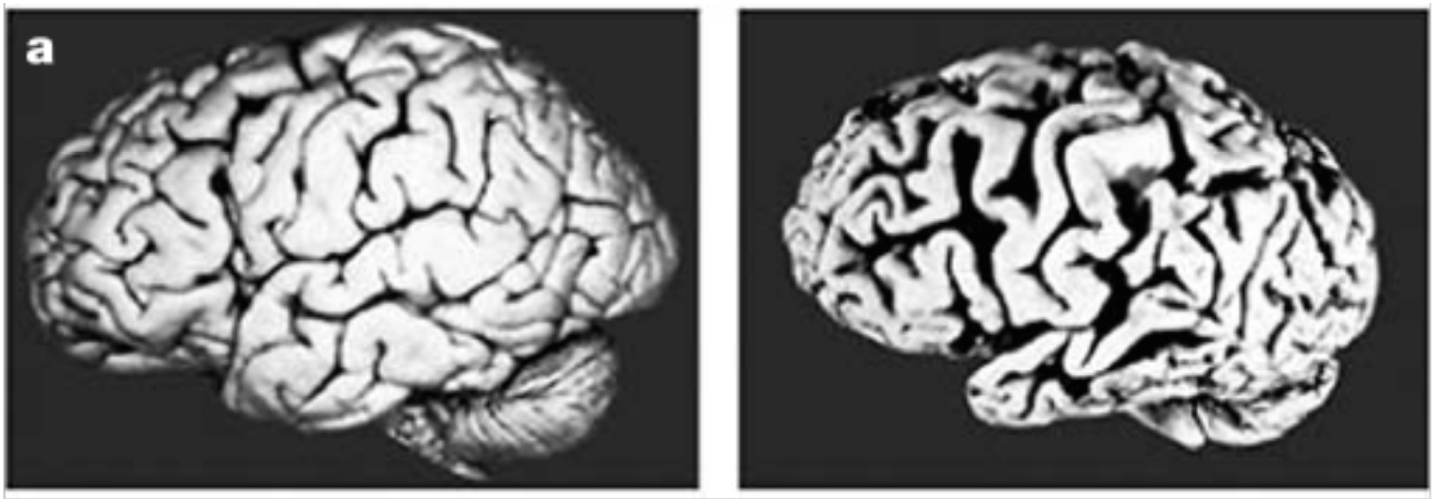
  - progressive and irreversible

- No current cure for AD.

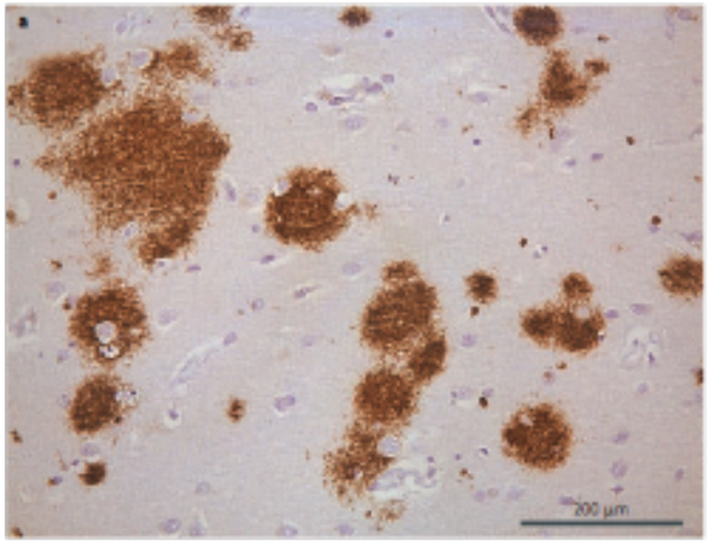
- Characterized pathologically by the accumulation / deposition of amyloid β (Aβ) and neurofibrillary tangles.

Normal

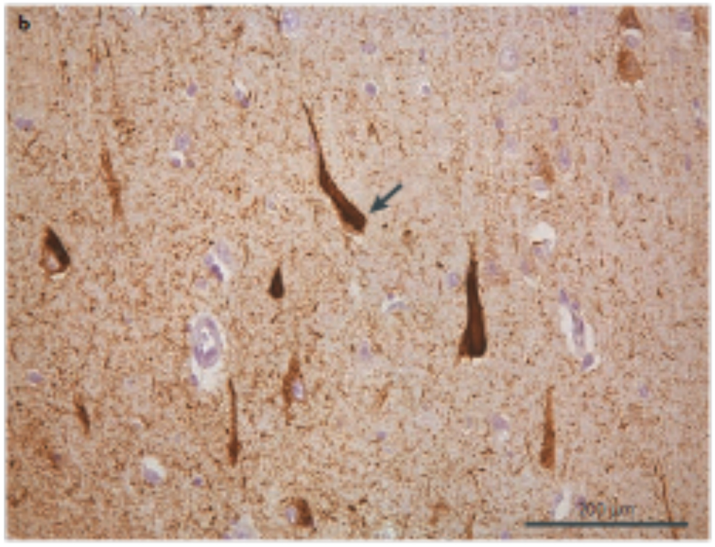
Alzheimer's



Senile plaques (Aβ)

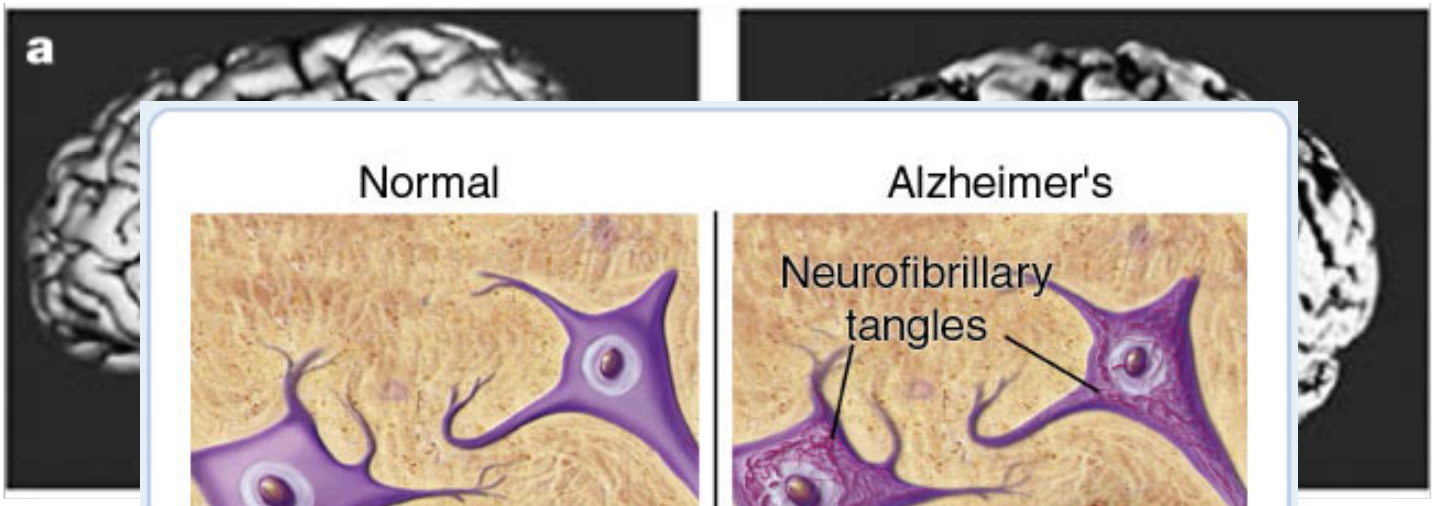


Neurofibrillary tangles (tau)



Normal

Alzheimer's



Sen

s (tau)

Alzheimer's Disease (AD)
Human Genetics

- "Early-onset" Alzheimer's is a rare form of the disease.
  - occurs in people age 30 to 60.
  - represents less than 5% of all people who have AD.
  - Most cases of early-onset AD are familial AD, caused by changes in one of three known genes:
    - *amyloid precursor protein* (*APP*) in chromosome 21
    - *presenilin 1* (*PS1*) in chromosome 14
    - *presenilin 2* (*PS2*) in chromosome 11

- Most people with AD have "late-onset" Alzheimer's.
  - usually develops after age 60.
  - Several genes, including *apolipoprotein E* (*APOE*; especially *APOE ε4* allele) and *TREM2*, may increase a person's risk for late-onset AD.
  - environmental factors???

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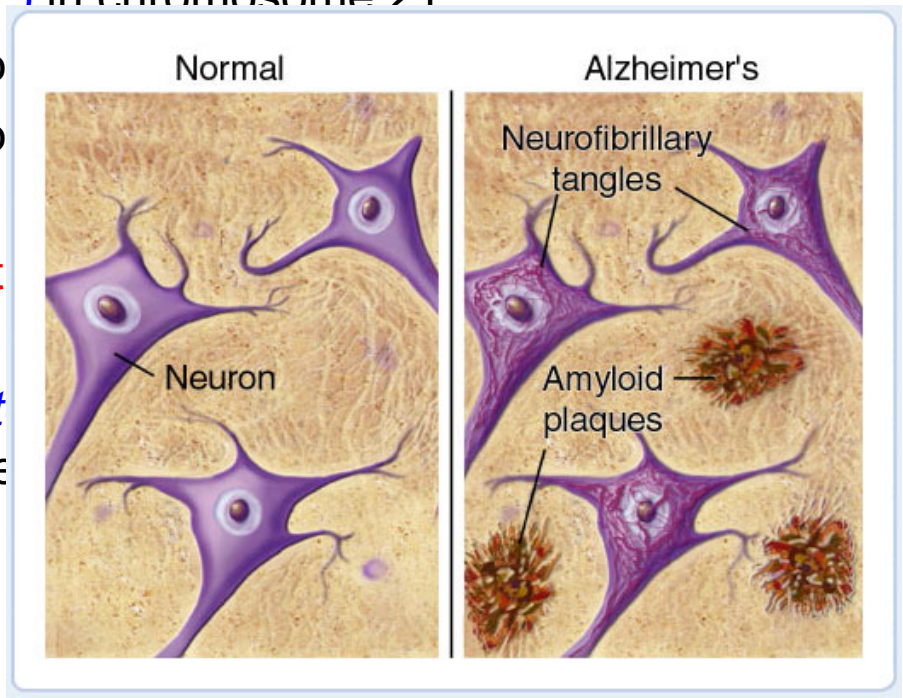
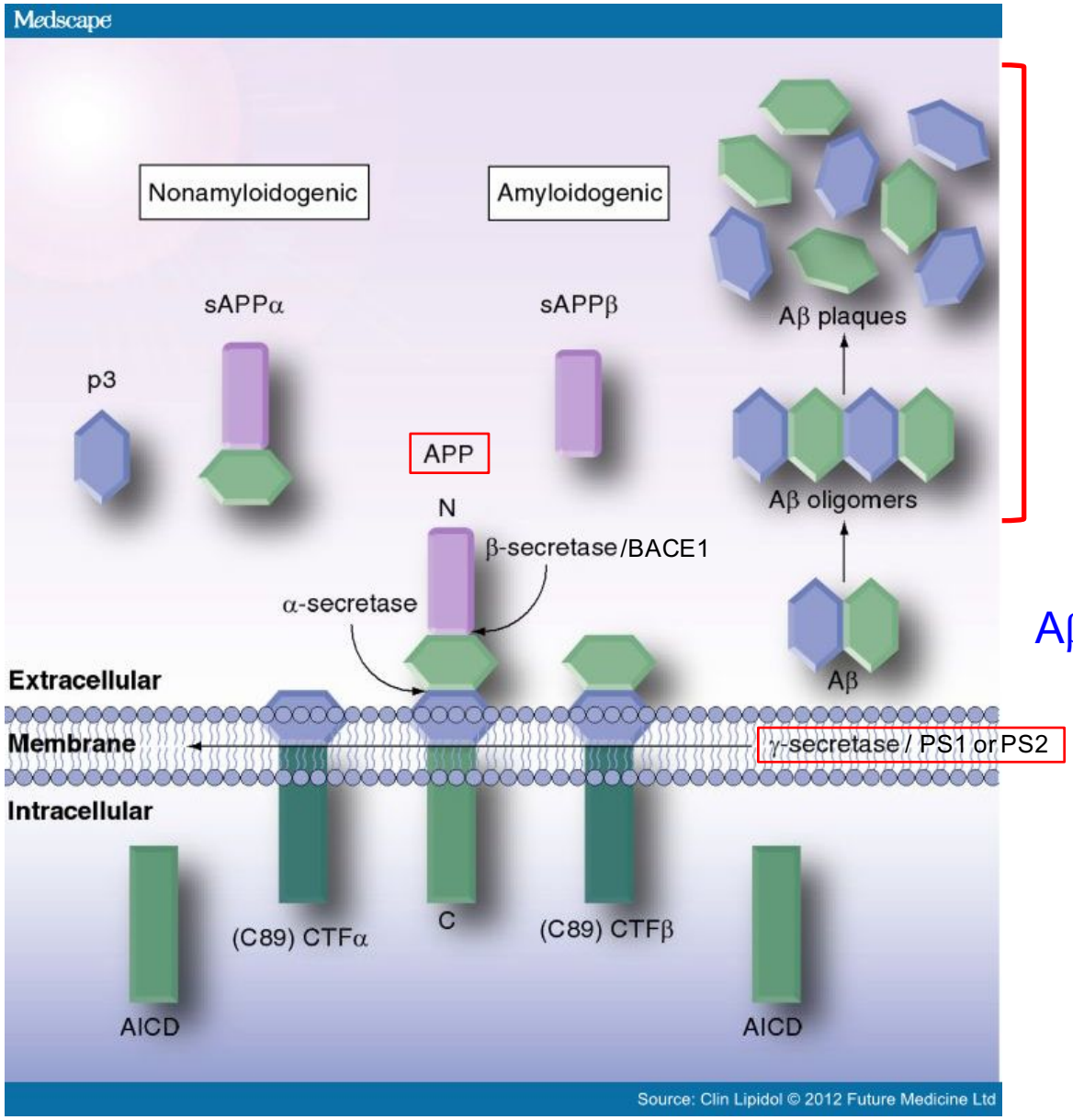


Proteolytic processing of APP



Amyloid plaques,
Cell death,
Synaptic dysfunction,
Other features of AD

Aβ40 or Aβ42

Lütjohann et al., Clin Lipidology, 2012

Aβ40 vs. Aβ42

- The γ secretase can generate a number of isoforms of 36-43 amino acid residues in length.

- The most common isoforms are $A\beta_{40}$ and $A\beta_{42}$.

  - The $A\beta_{40}$ form is the more common of the two.

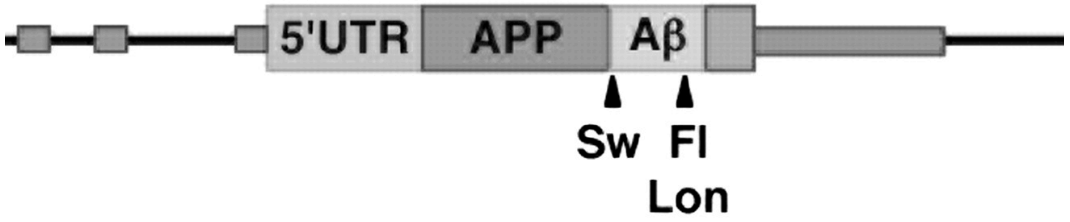
  - $A\beta_{42}$ is the more fibrillogenic and is thus associated with disease states.

  - Mutations in APP associated with early-onset Alzheimer's have been noted to increase the relative production of $A\beta_{42}$.

  - Aβ is destroyed by several amyloid-degrading enzymes, including neprilysin.

Familial Alzheimer's disease (FAD) mutations

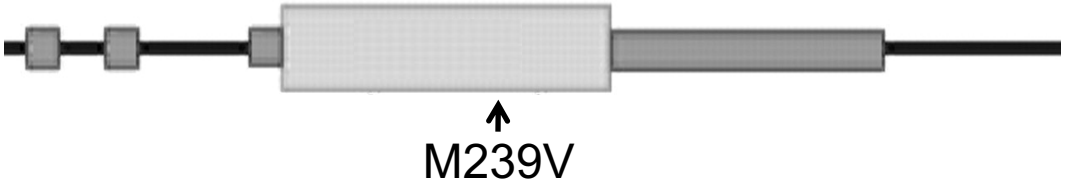
- APP



- Sw (Swedish mutation, M671L), Fl (Florida mutation, I716V), Lon (London mutation, V717IL), etc.
- More than 50 different mutations known in APP, causing FAD.

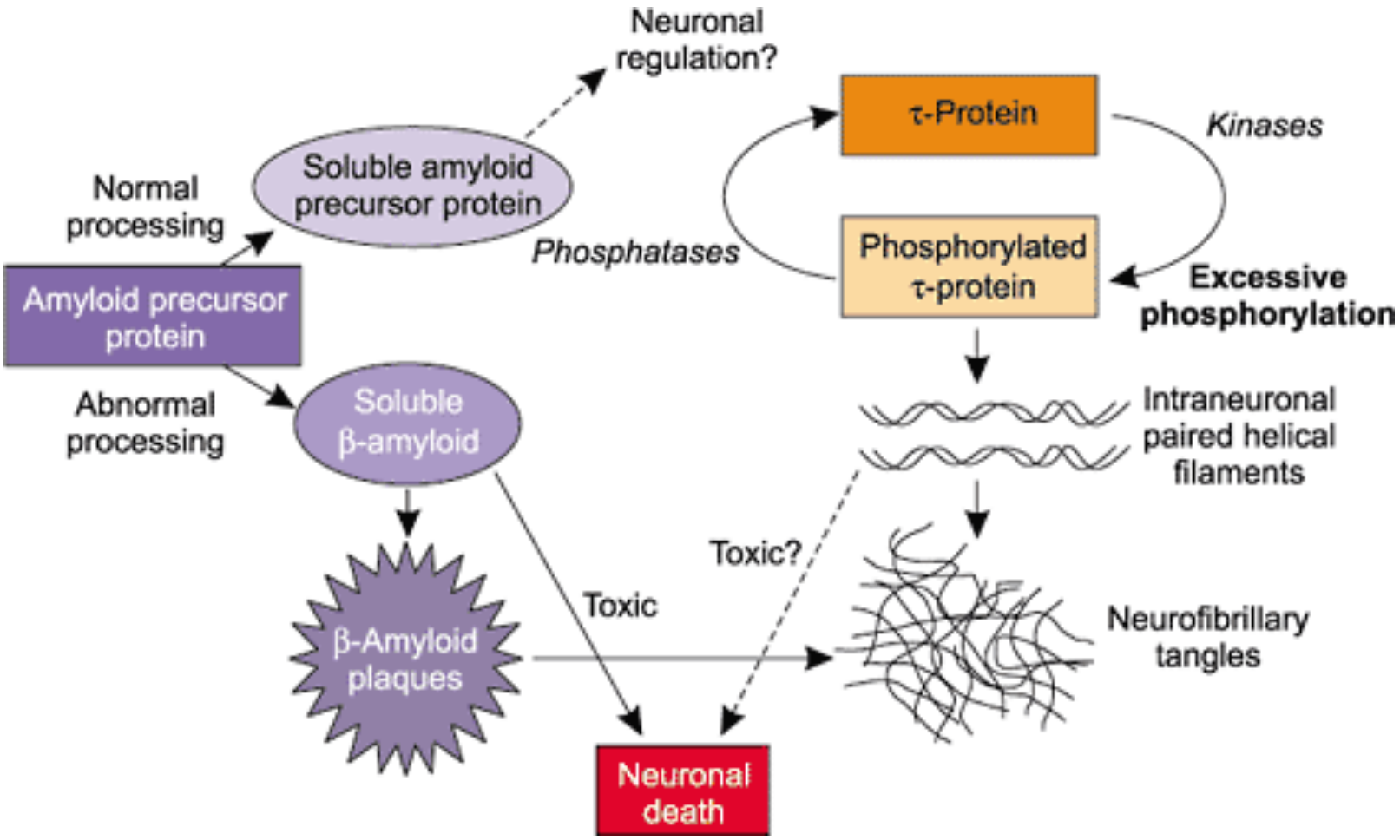
- PS1

- PS2



- Mutations in PS1 or PS2 cause substantial changes in the Aβ42/Aβ40 ratio.

Two pathological hallmarks of AD



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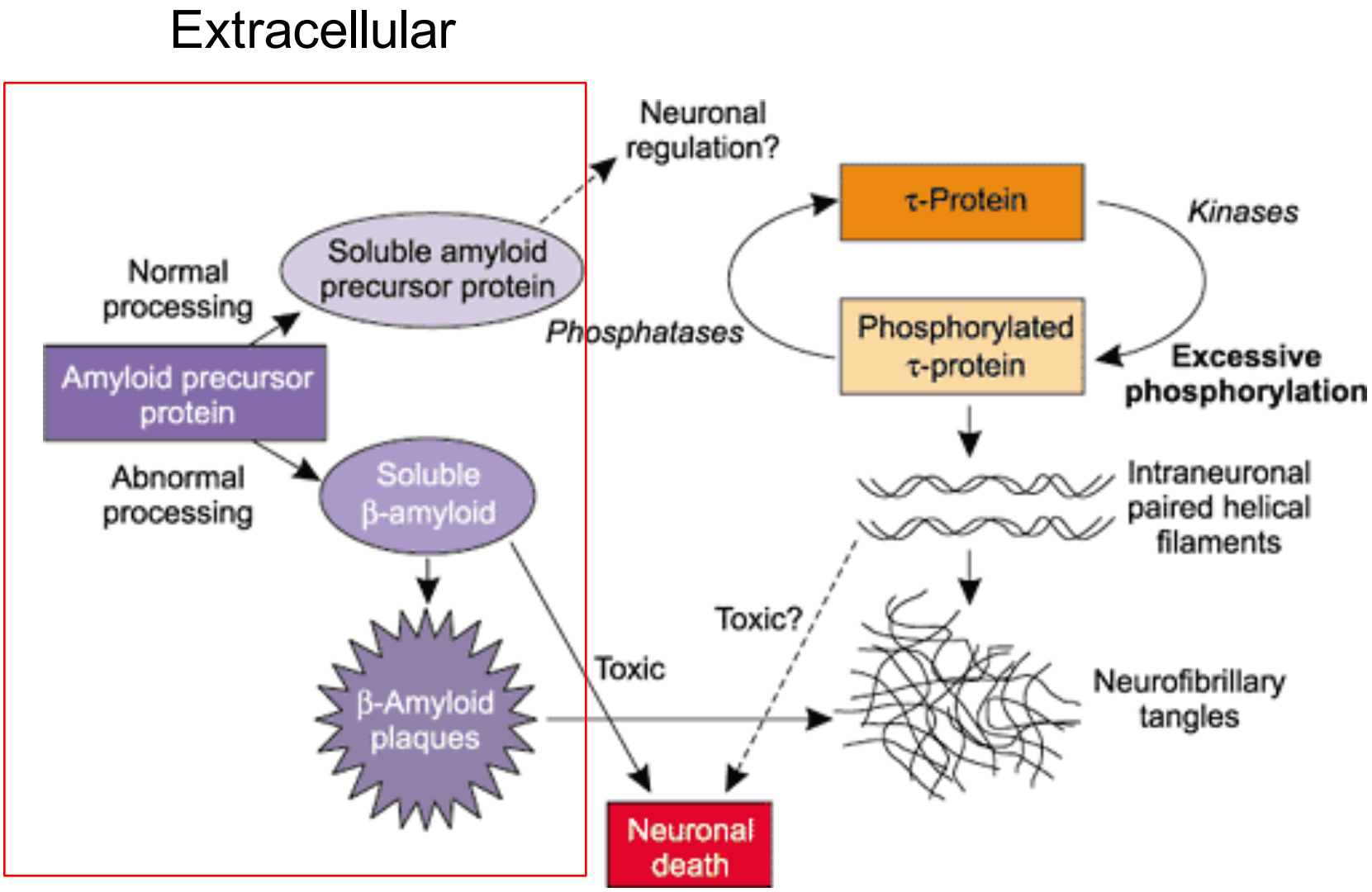

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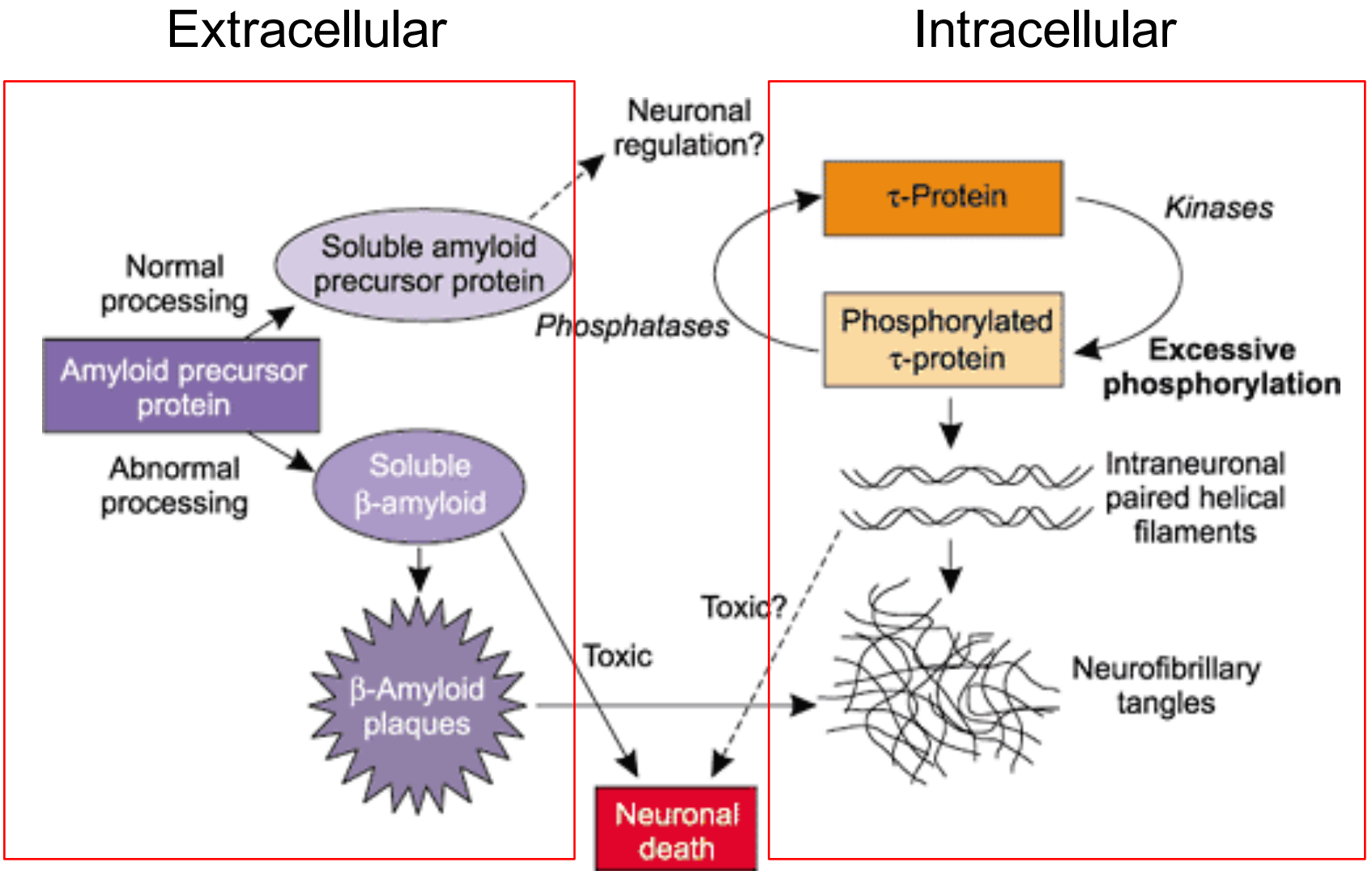


Amyloid hypothesis of AD

Amyloid cascade hypothesis

Changes in Aβ metabolism

Oligomerization of Aβ42 and diffuse plaque deposits

Subtle effects of soluble oligomers on synapse function

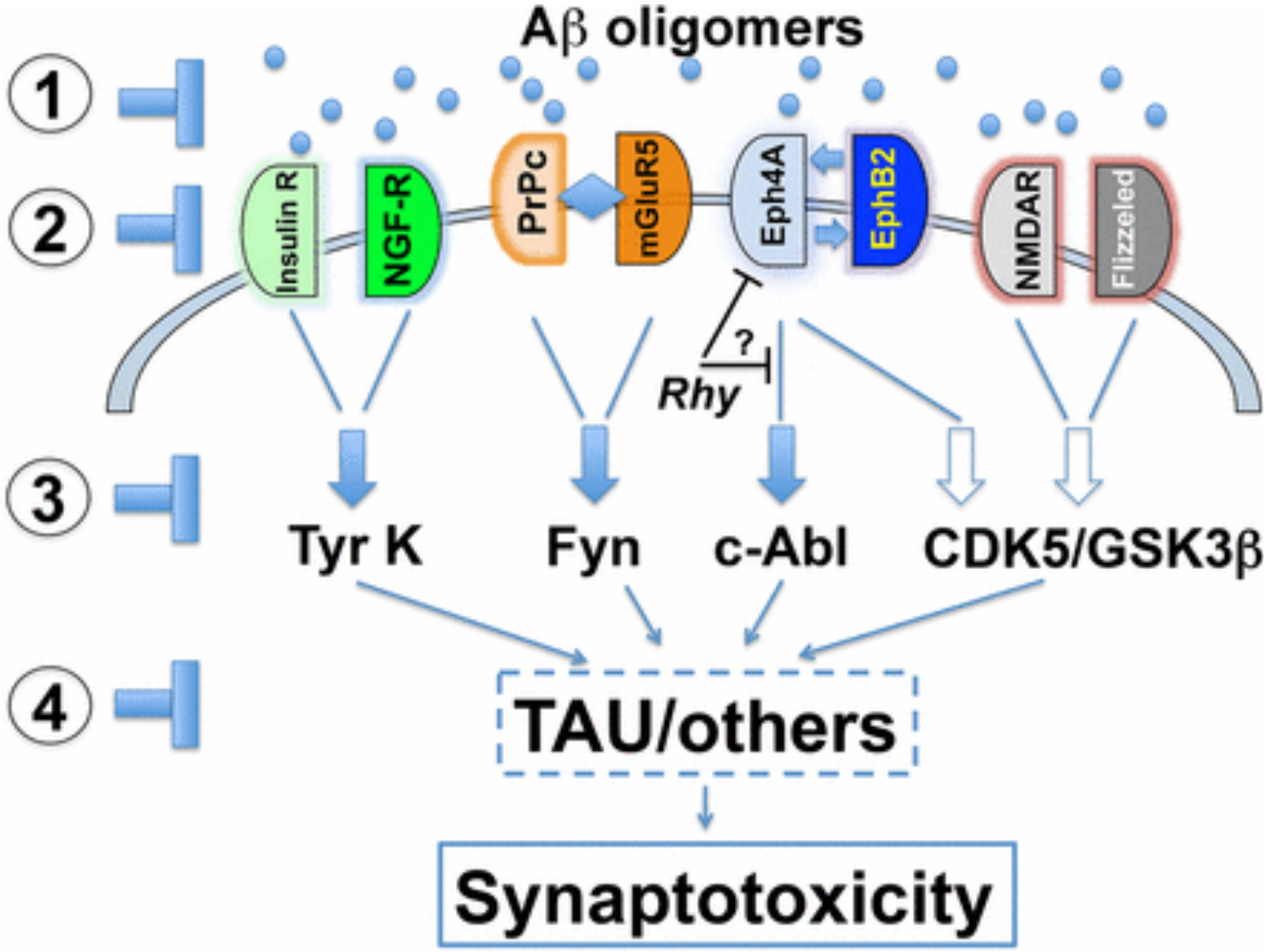
Abnormal distribution of tau and aberrant tau phosphorylation

Oligomerization of tau

Propagation and neuronal dysfunction leading to cell death

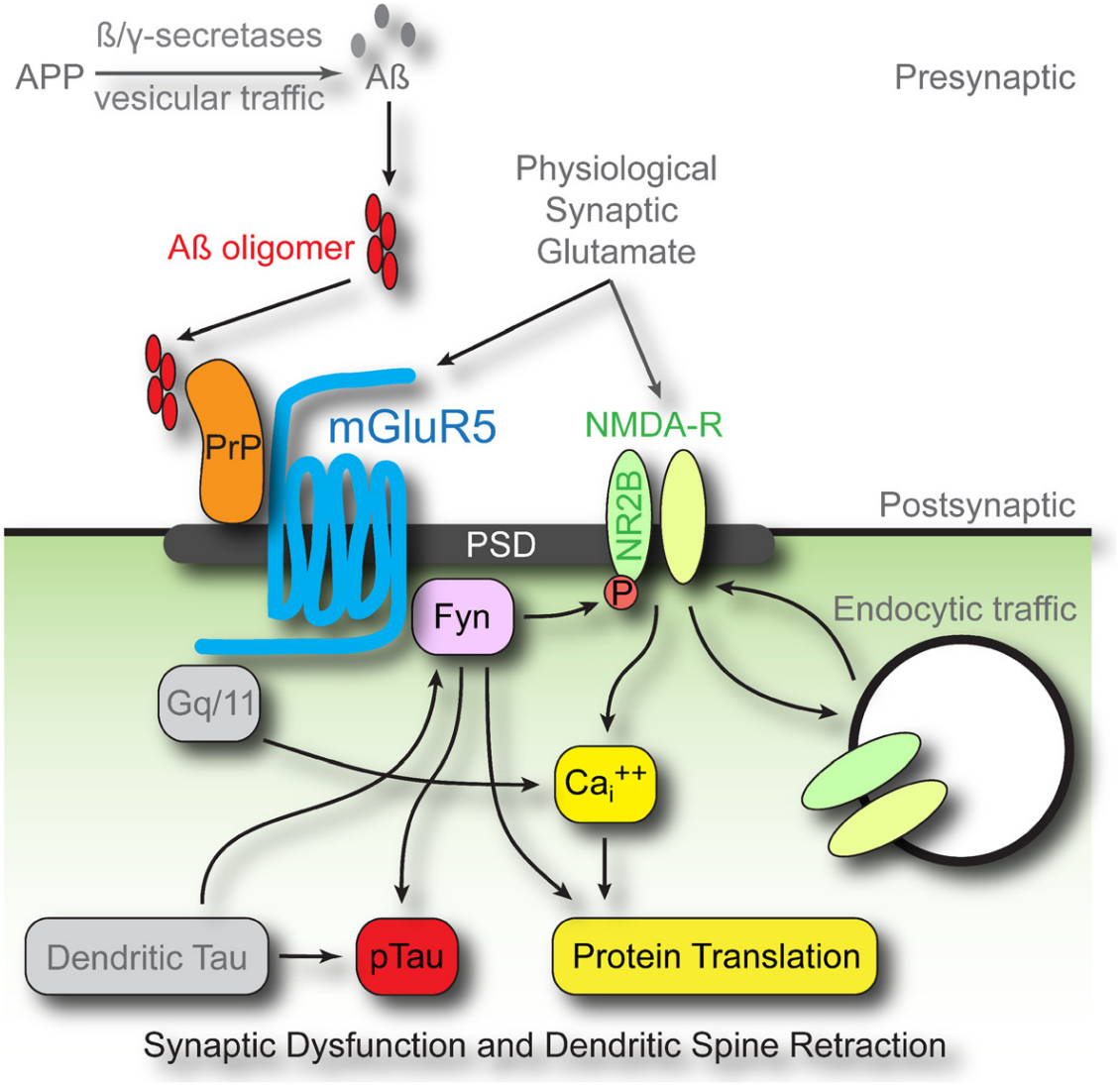
Dementia with plaque and tangle pathology

Putative Aβ oligomer receptors, signaling pathways, and therapeutic targets



Overk and Masliah, *PNAS*, 2014

A model for Aβo-PrP-mGluR causing AD-related phenotypes



Nygaard et al.,Alzheimers Res Ther, 2011

A beneficial mutation in APP against AD

LETTER

96 | NATURE | VOL 488 | 2 AUGUST 2012

doi:10.1038/nature11283

A mutation in *APP* protects against Alzheimer's disease and age-related cognitive decline

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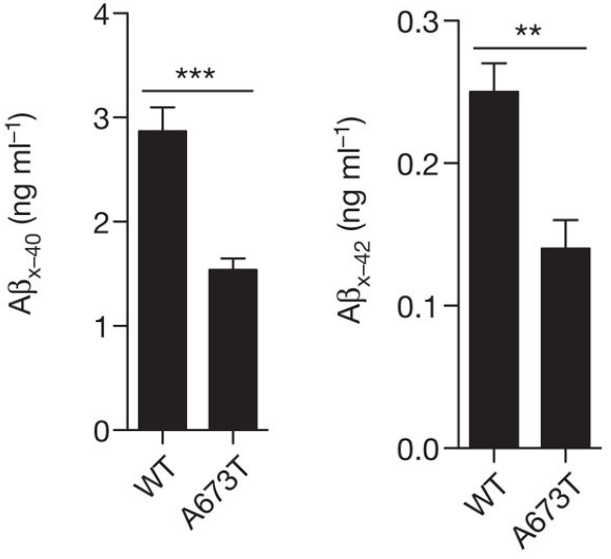
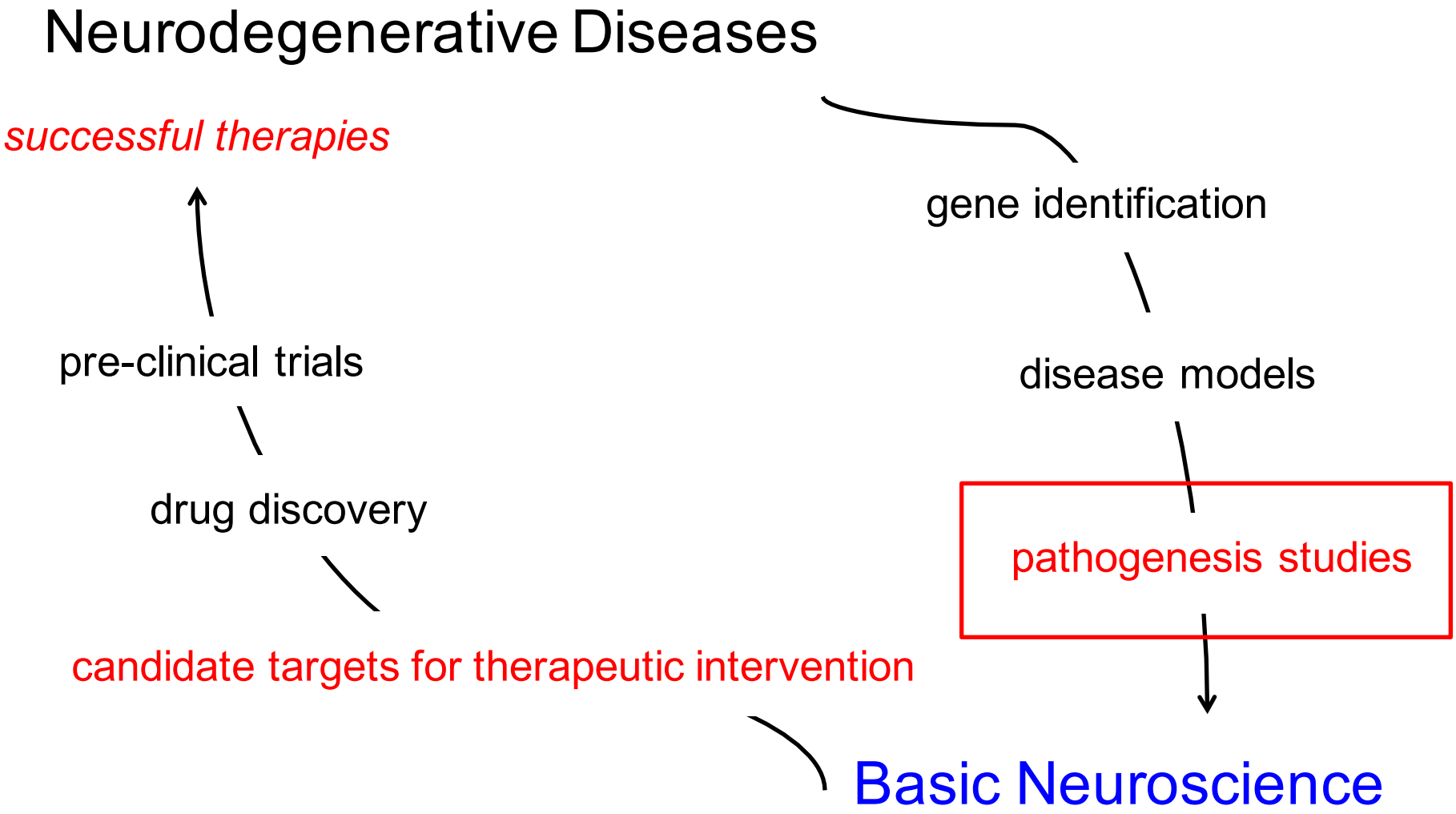
- 1,795 Icelanders who had lived to at least age 85 without a diagnosis of Alzheimer's disease.
  >>> Whole genome sequence.

- A coding mutation (A673T) in the *APP* gene.
  - significantly more common in the elderly control group than in the Alzheimer's disease group (0.62% versus 0.13%; odds ratio (OR) = 5.29; *P value* = 4.78×10^{-7}).

- A673T substitutions reduces BACE1 cleavage of APP relative to wild-type APP.



Translational Neuroscience



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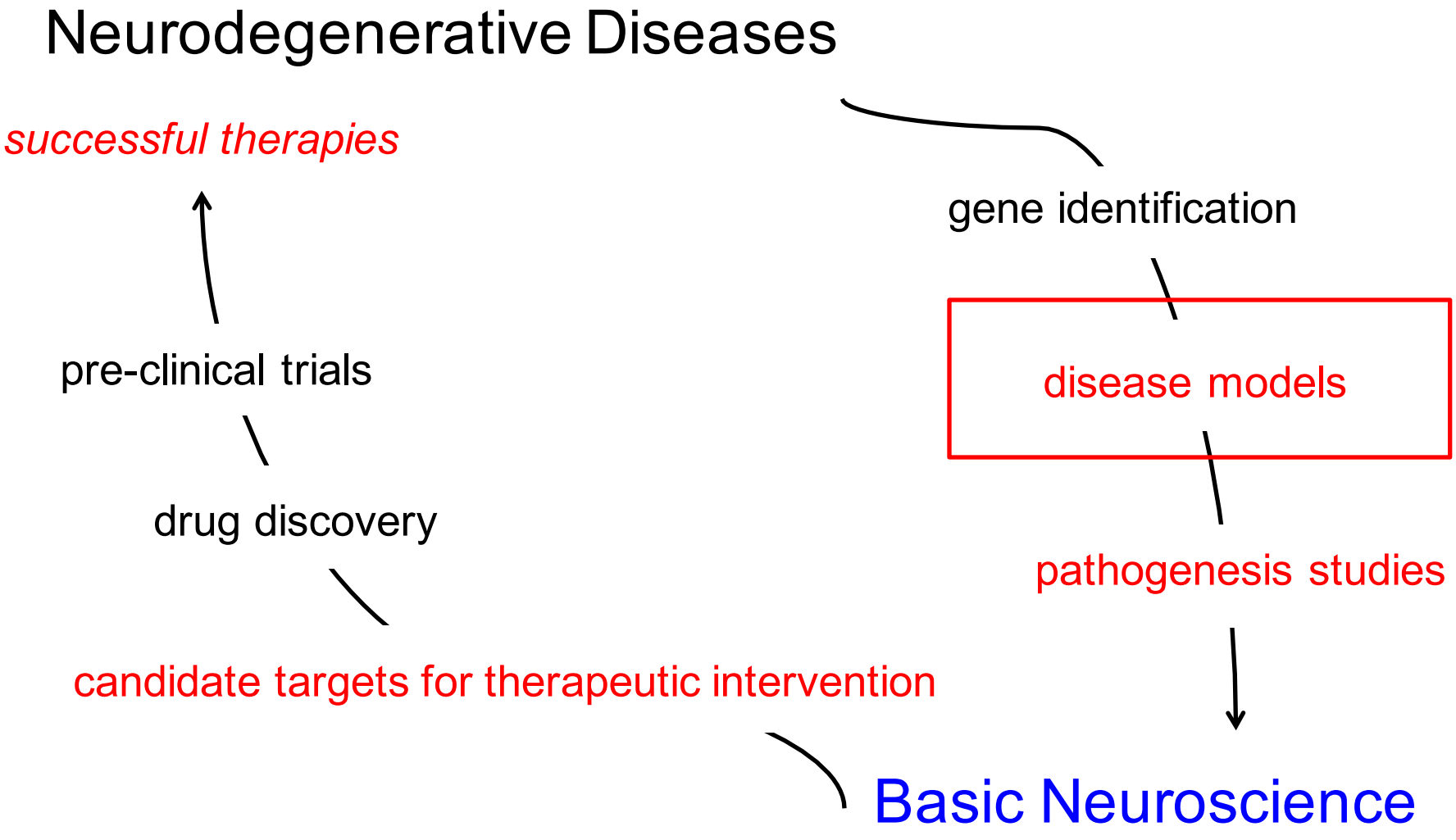

Summary of selected mouse models used in Alzheimer's disease research

Transgenic mouse	Transgene (mutation)[a]	Promoter	Strains	Amyloid plaques	NFTs	Neuron loss	Cognitive deficits	Primary references
PDAPP	APP695, 751, 770 (APPInd)	PDGF-β	C57Bl/6 J, DBA/2, Swiss-Webster	6–9 months	No	No	Yes	(20)
Tg2576	APP695 (APPSwe)	Hamster PrP	C57Bl/6SJL, C57Bl/6	9 months	No	No	Yes	(21)
APP23	APP751 (APPSwe)	Mouse Thy-1	C57Bl/6, DBA/2	6 months (severe CAA also present)	No	14 months	Yes	(26)
J20[b]	APP695, 751, 770 (APPSwe, Ind)	PDGF-β	C57Bl/6	6 months	No	No	Yes	(23)
TgCRND8	APP695 (APPSwe, Ind)	Hamster PrP	C3H, C57Bl/6	3 months	No	No	Yes	(28)
mThy1-hAPP751	APP695 (APPSwe, Lon)	Mouse Thy-1	C57Bl/6, DBA/2	3–4 months	No	No	Yes	(29)
APPDutch	APP751 (APPDutch)	Mouse Thy-1	C57Bl/6 J	22 months (CAA only)	No	Not reported	Not reported	(30)
ARC6, ARC48	APP695, 751, 770 (APPSwe, Ind, Arc)	PDGF-β	C57Bl/6	3 months (6) 2 months (48)	No (6) No (48)	No (6) No (48)	No (6) Yes (48)	(31)
PSAPP	Tg2576 X PSEN1-M146L	Hamster PrP PDGF-β	C57Bl/6SJL, C57Bl/6 B6D2F1, Swiss-Webster	6 months	No	No	Yes	(35)
5XFAD	APP695 (Swe, Lon, Flo) PSEN1-M146L, L286V	Mouse Thy-1 Mouse Thy-1	C57Bl/6SJL	2 months	No	9 months	Yes	(37)

(Continued)

Chin J, Methods Mol. Bio., 2011

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ARC6, ARC48	APP695, 751, 770 (APPSwe, Ind, Arc)	PDGF-β	C57Bl/6	3 months (6) 2 months (48)	No (6) No (48)	No (6) No (48)	No (6) Yes (48)	(31)
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(Continued)

Chin J, Methods Mol. Bio., 2011

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APP23	APP751 (APPSwe)	Mouse Thy-1	C57Bl/6, DBA/2	6 months (severe CAA also present)	No	14 months	Yes	(26)
J20[b]	APP695, 751, 770 (APPSwe, Ind)	PDGF-β	C57Bl/6	6 months	No	No	Yes	(23)
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- There is no single mouse model that perfectly recapitulates the pathology seen in patients with Alzheimer's disease.

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3XTG[d]	APP695 (Swe) 4R0N MsAPT (P301L) PSEN1-M146V (Knock-in)	Mouse Thy-1.2 Mouse Thy-1.2	129/C57Bl/6	6 months	12 months	No	Yes	(50)

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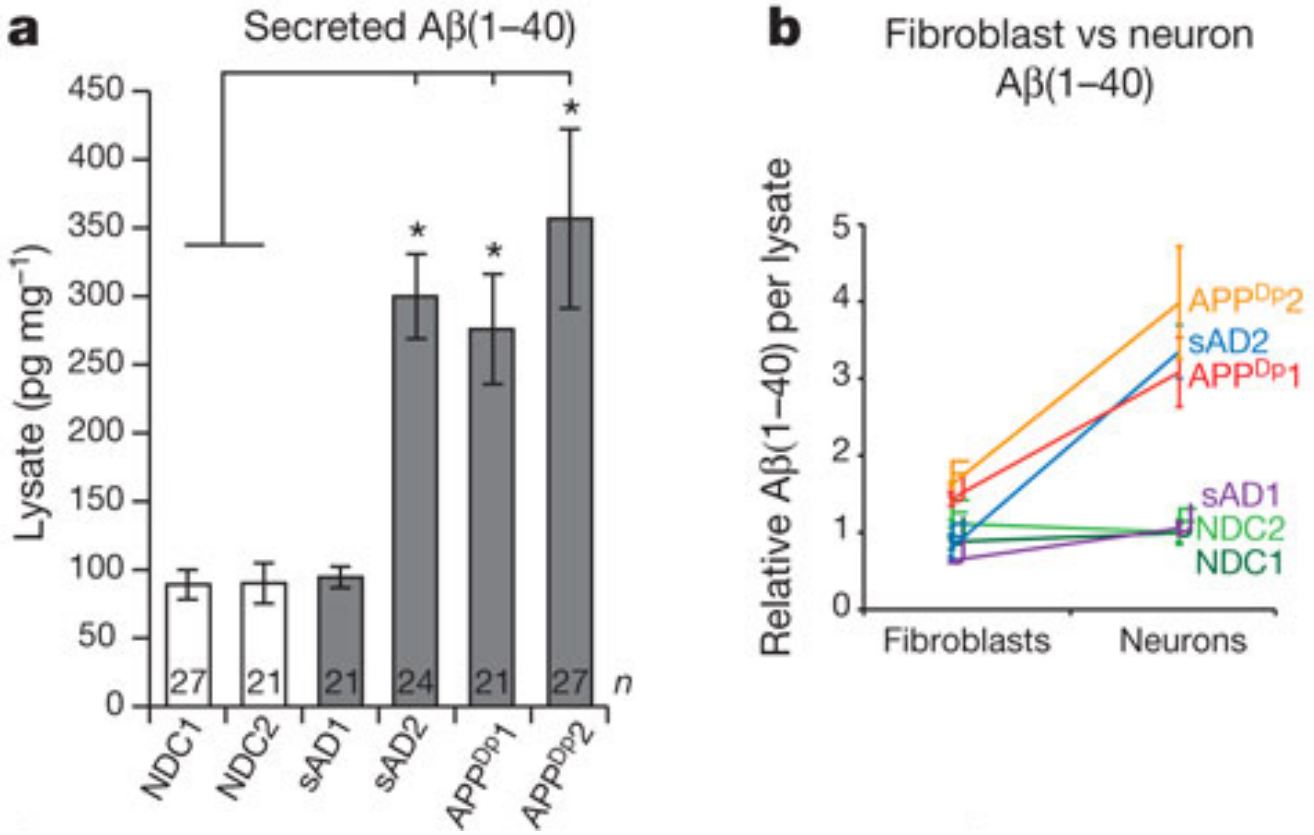
Cell culture models for Alzheimer's disease research

Cell culture models for Alzheimer's disease research

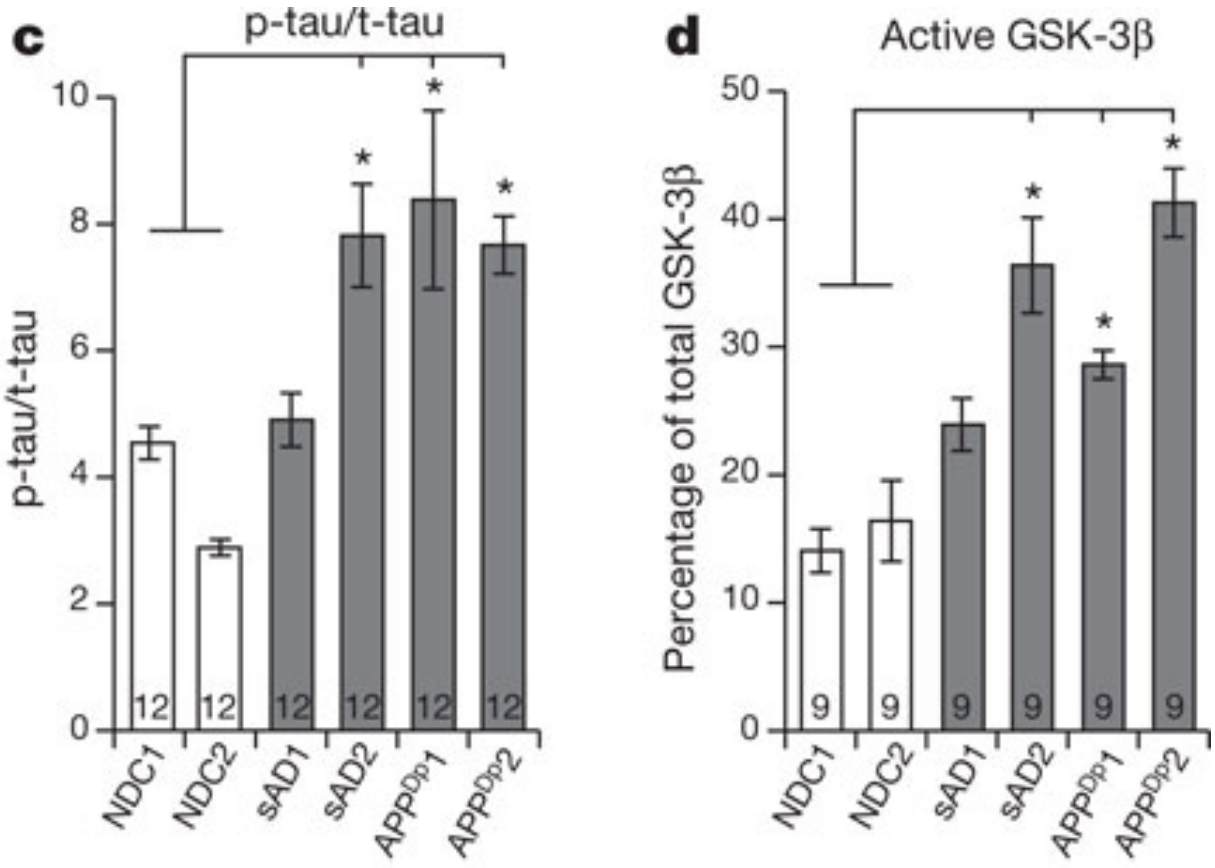
- Is it any good for Alzheimer's disease research?

Increased amyloid-β production in neurons derived from AD-patient-derived iPSCs



Israel et al., Nature, 2012

Increased phospho-tau and active GSK-3β in neurons derived from AD-patient-derived iPSCs



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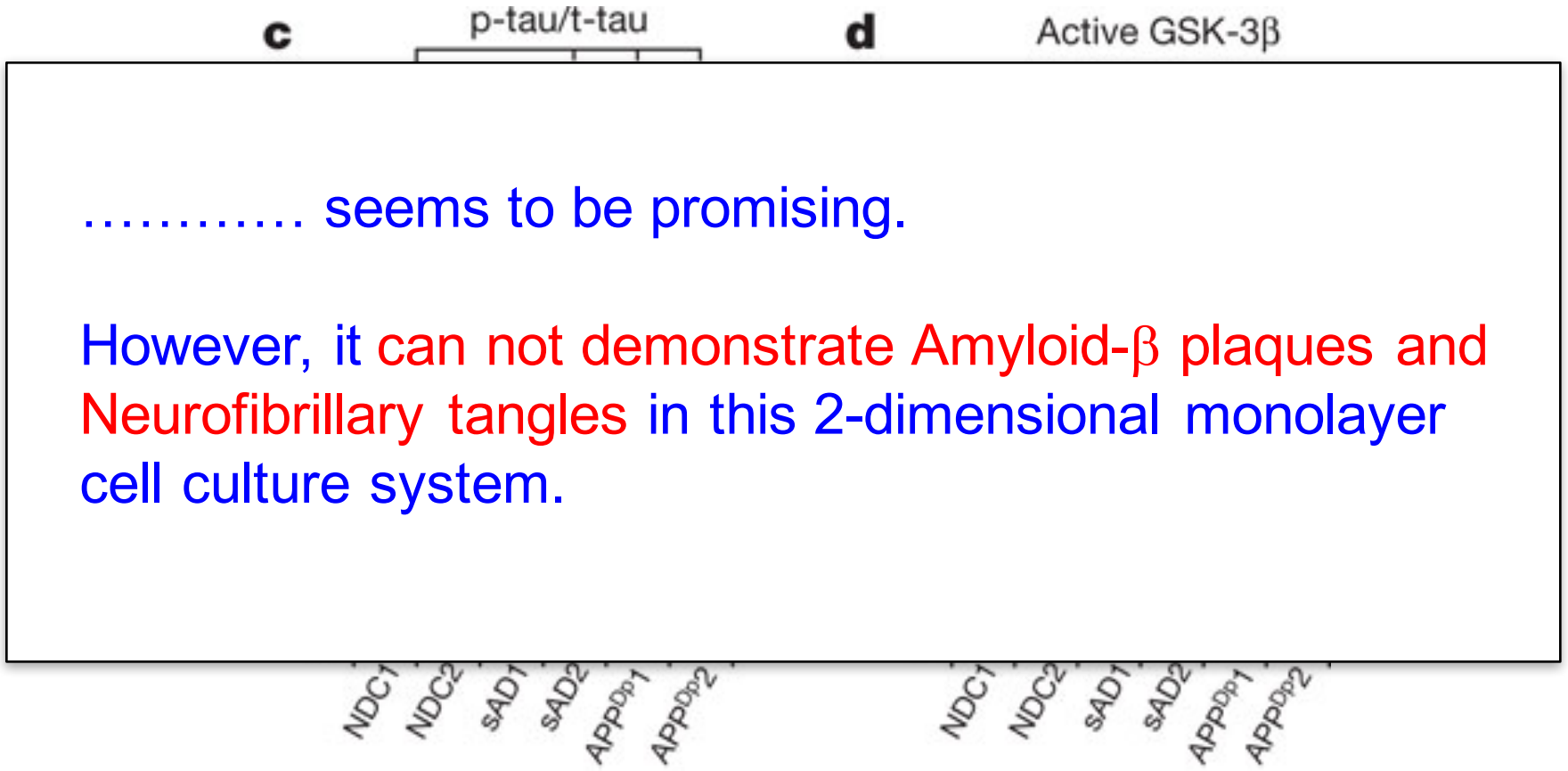


…… seems to be promising.

However, it can not demonstrate Amyloid-β plaques and Neurofibrillary tangles in this 2-dimensional monolayer cell culture system.

Israel et al., Nature, 2012

LETTER

00 MONTH 2014 | VOL 000 | NATURE | 1

doi:10.1038/nature13800

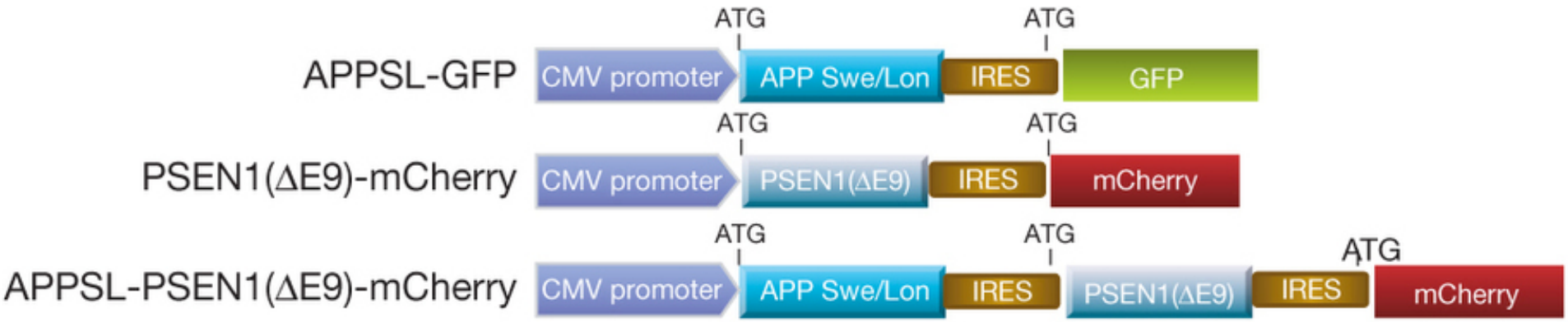
A three-dimensional human neural cell culture model of Alzheimer's disease

Se Hoon Choi[1]*, Young Hye Kim[1,2]*, Matthias Hebisch[1,3], Christopher Sliwinski[1], Seungkyu Lee[4], Carla D'Avanzo[1], Hechao Chen[1], Basavaraj Hooli[1], Caroline Asselin[1], Julien Muffat[5], Justin B. Klee[1], Can Zhang[1], Brian J. Wainger[4], Michael Peitz[3], Dora M. Kovacs[1], Clifford J. Woolf[4], Steven L. Wagner[6], Rudolph E. Tanzi[1] & Doo Yeon Kim[1]

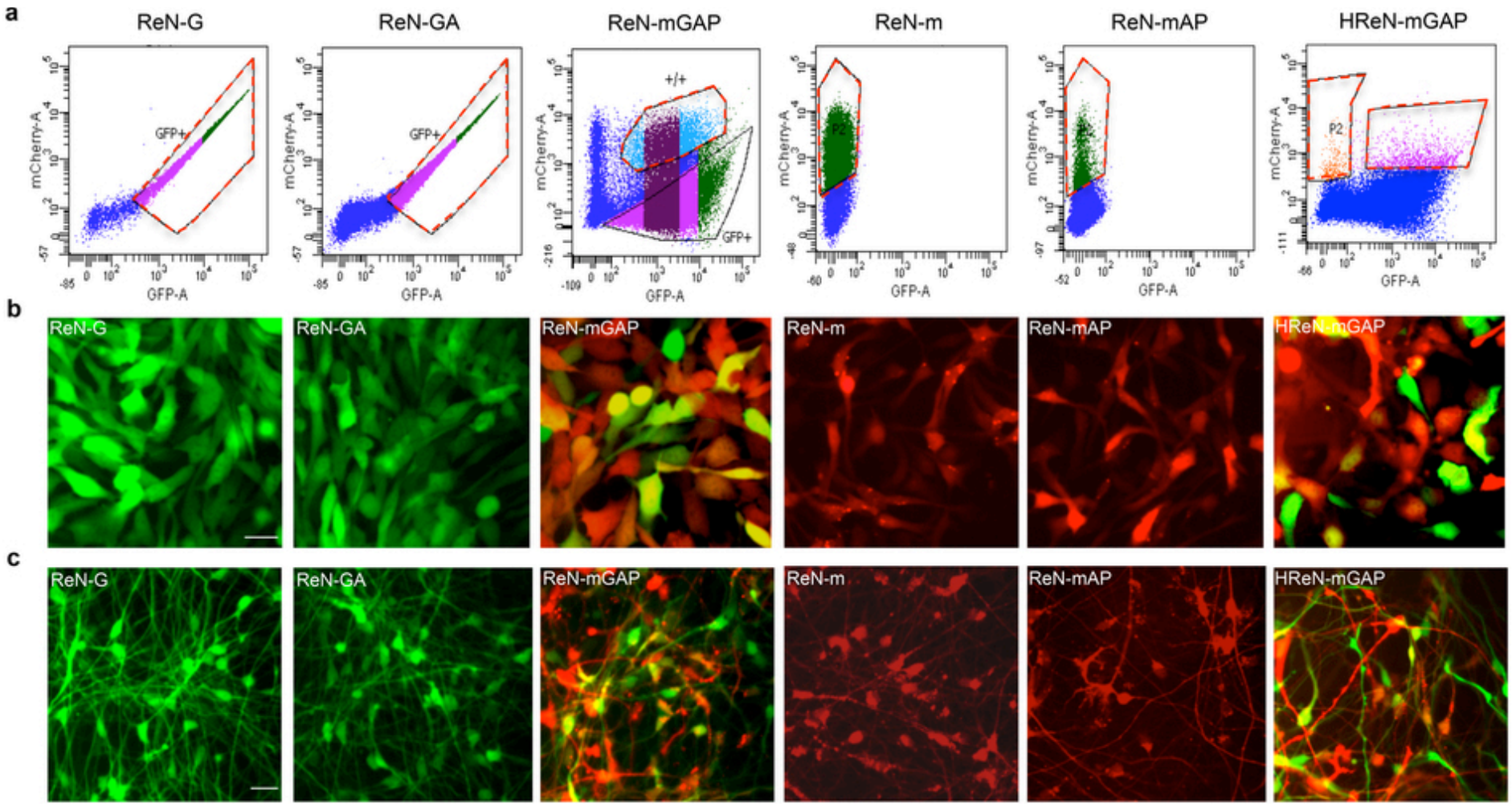
Generation of hNPCs with multiple FAD mutations

- Human neural progenitor cells (hNPCs).
- Overexpress human *APP* and *PSEN1* genes, containing FAD mutations.

Lentiviral vectors:

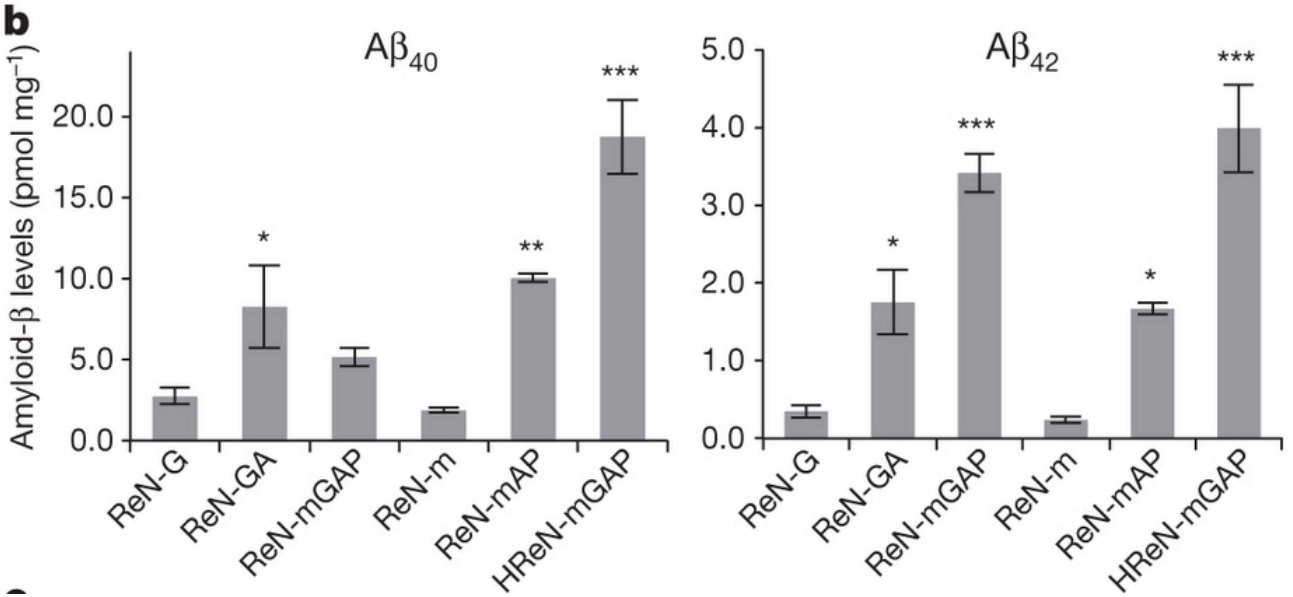


FACS sorting of hNPCs and their differentiation



- Most ReN stem cells differentiated into neuronal and glial cells within 3 weeks.

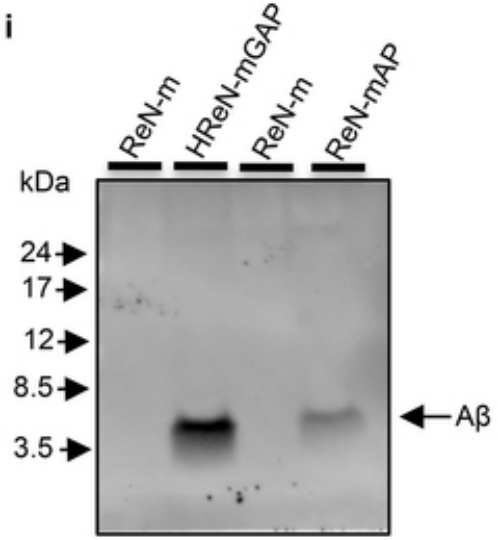
Increased amyloid-β levels in conditioned media



Aβ42: ~17-fold increase

Aβ40: ~9-fold increase

Aβ42 / Aβ40: ~ 2-fold



6-week differentiated condition

f

Type	Cell line name	Genes overexpressed
Control	ReN-G	GFP
	ReN-m	mCherry
	ReN-m (enriched)	mCherry
FAD	ReN-GA	APPSL/GFP
	ReN-mGAP	APPSL/GFP + PSEN1(ΔE9)/mCherry
	HReN-mGAP	GFP + APPSL/PSEN1(ΔE9)/mCherry
	ReN-mAP	APPSL/PSEN1(ΔE9)/mCherry
	ReN-mAP (enriched)	APPSL/PSEN1(ΔE9)/mCherry

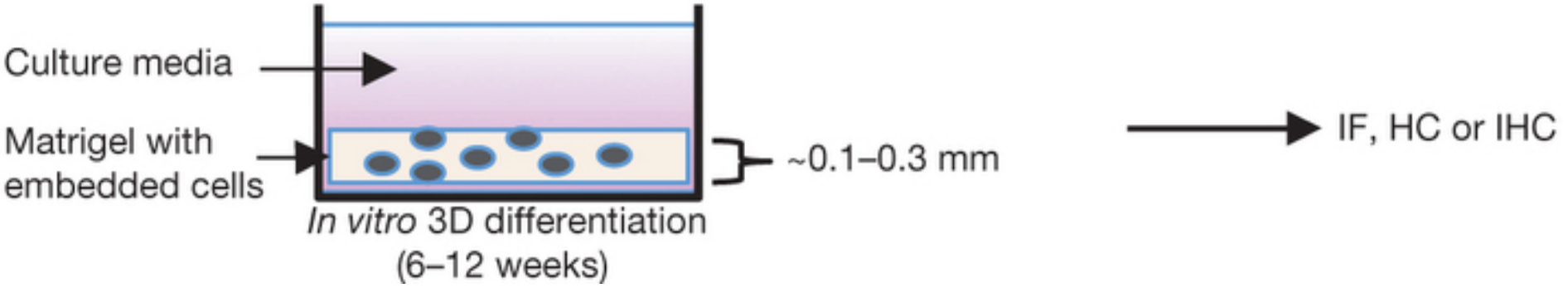
In conventional 2D cultures, secreted amyloid-β diffuses into a large volume of media.

Therefore, they hypothesized that a 3D culture would accelerate amyloid-β deposition by limiting diffusion of amyloid-β, allowing for aggregation.

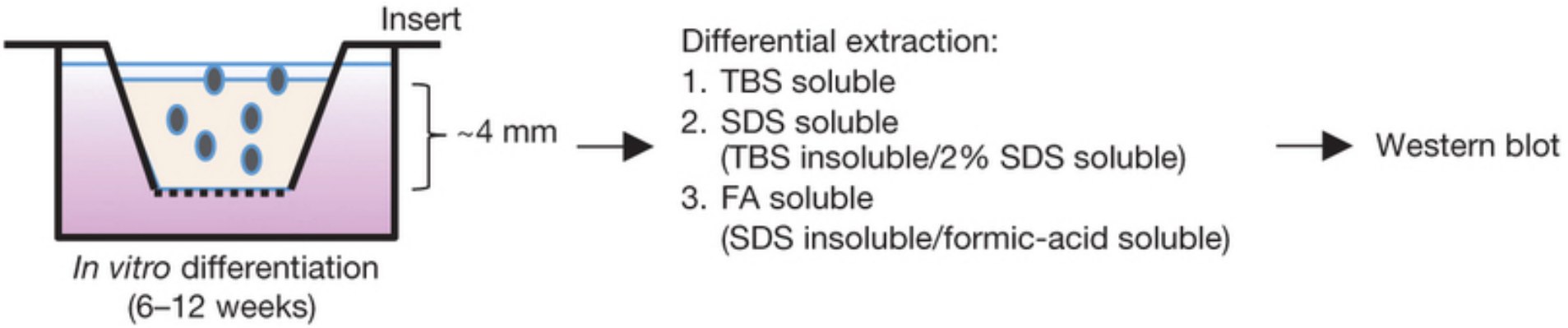
3D cell culture models

- BD Matrigel (BD Biosciences) as a 3D support matrix since it contains high levels of brain extracellular matrix proteins.

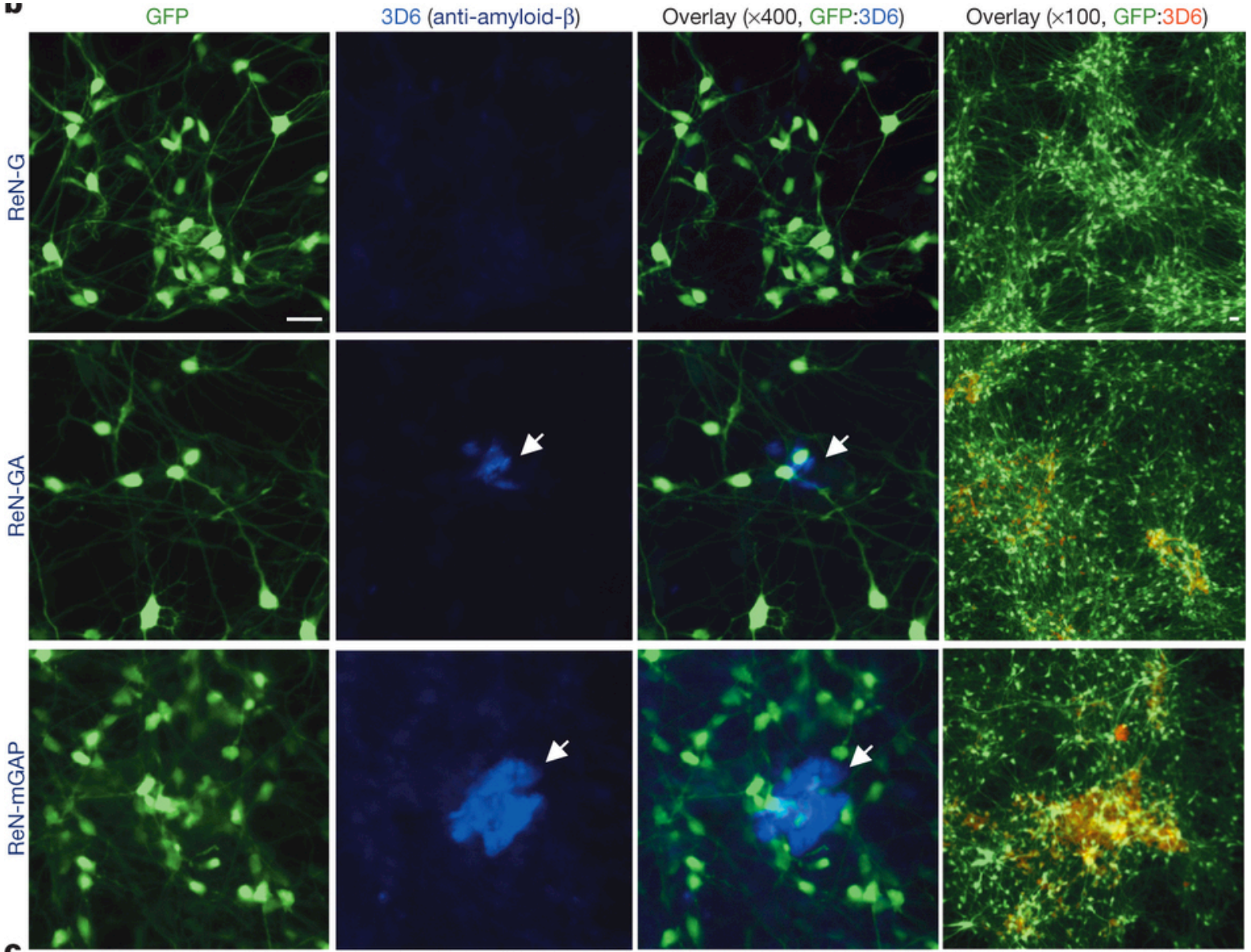
- Thin-layer 3D culture protocol:



- Thick-layer 3D culture protocol:



Robust increase of extracellular amyloid-β deposits in 3D-differentiated hNPCs with FAD mutations



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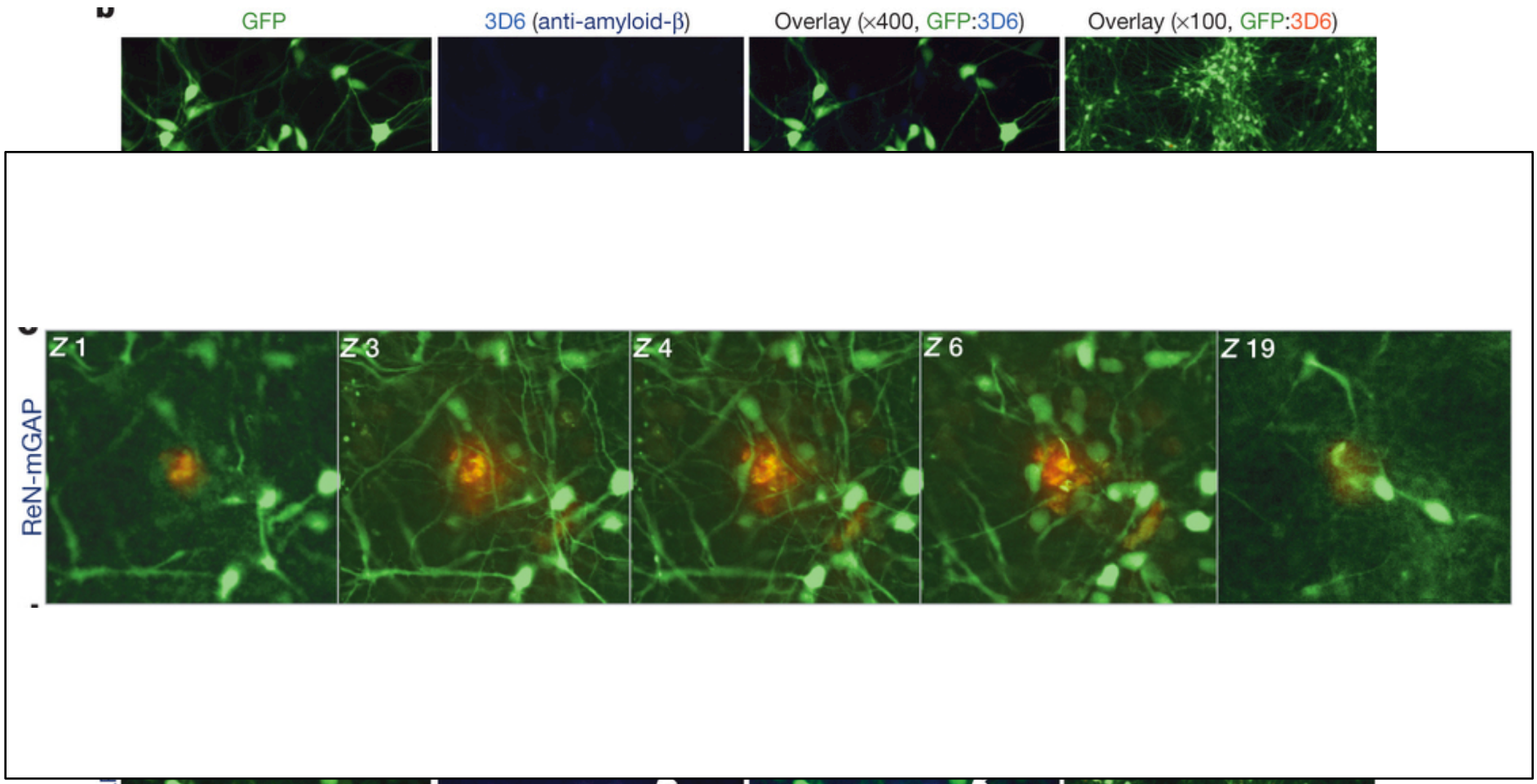

LETTER

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- Elevation of amyloid-β and p-tau shown by western blot.
- Elevated p-tau proteins are aggregated in a manner similar to those observed in the degenerating AD neurons.

Discussion

- Successfully recapitulates amyloid-β and tau pathologies of AD.

- Questions need to be addressed:

  - Does it cause neurodegeneration and/or synaptic dysfunction?

  - What is the role of glial cells in a 3D cell culture model of AD?

    i.e. Neuroinflammation vs. phagocytosis?

  - How does it closely mimic *in vivo* brain contexts?

    i.e. Organoid culture using iNeurons derived from sporadic or familial AD?

PLOS ONE

RESEARCH ARTICLE

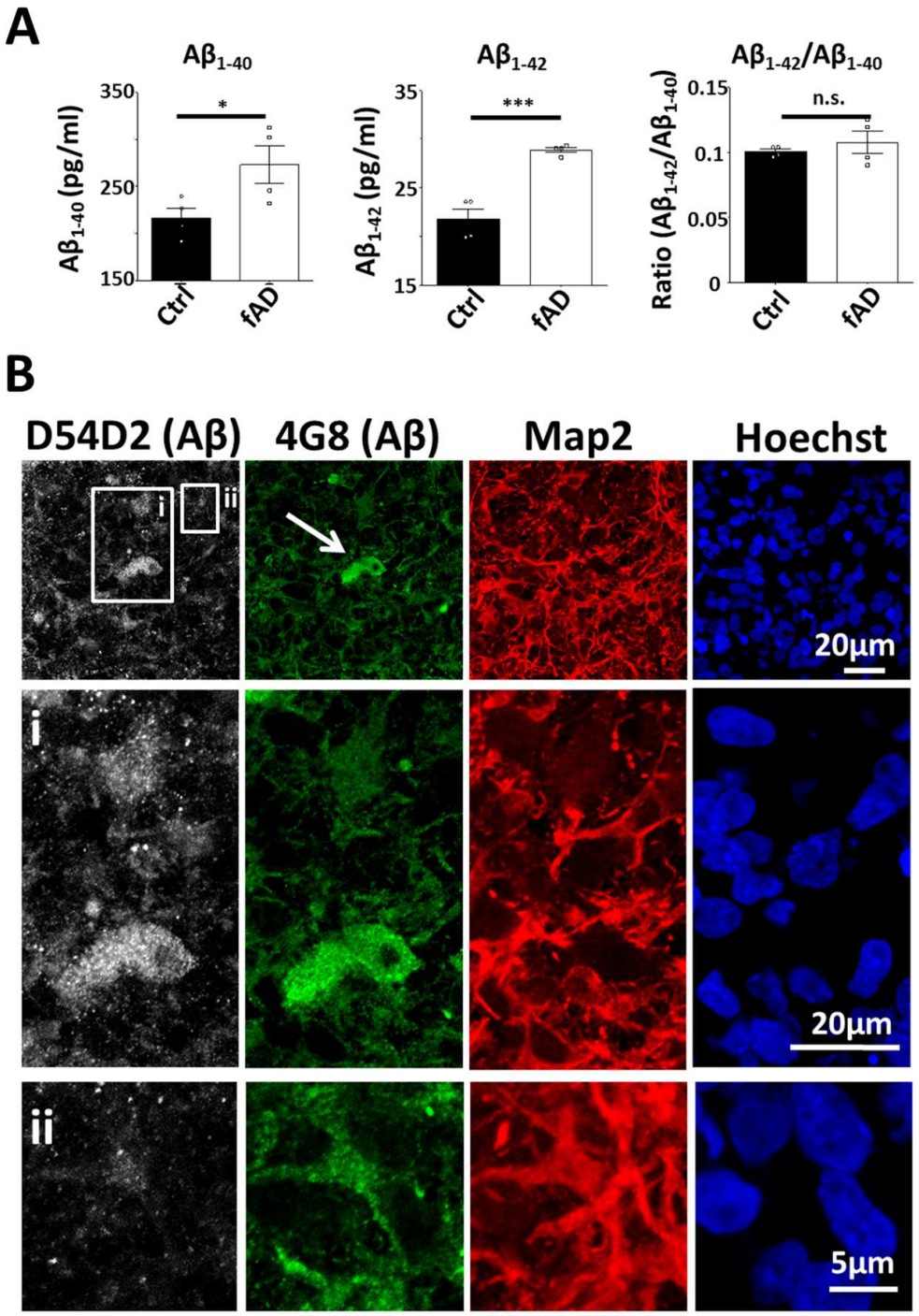
Self-Organizing 3D Human Neural Tissue Derived from Induced Pluripotent Stem Cells Recapitulate Alzheimer's Disease Phenotypes

Waseem K. Raja[1], Alison E. Mungenast[1], Yuan-Ta Lin[1], Tak Ko[2], Fatema Abdurrob[1], Jinsoo Seo[1], Li-Huei Tsai[1]*

1 Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, United States of America, **2** Induced Pluripotent Stem Cell Core Facility, Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, United States of America

* lhtsai@mit.edu

PLOS ONE



al Tissue
ıt Stem Cells
e Phenotypes

Fatema Abdurrob[1],

ive Sciences, Massachusetts
Pluripotent Stem Cell Core
f Technology, Cambridge, MA,

PLOS ONE

RESEARCH ARTICLE

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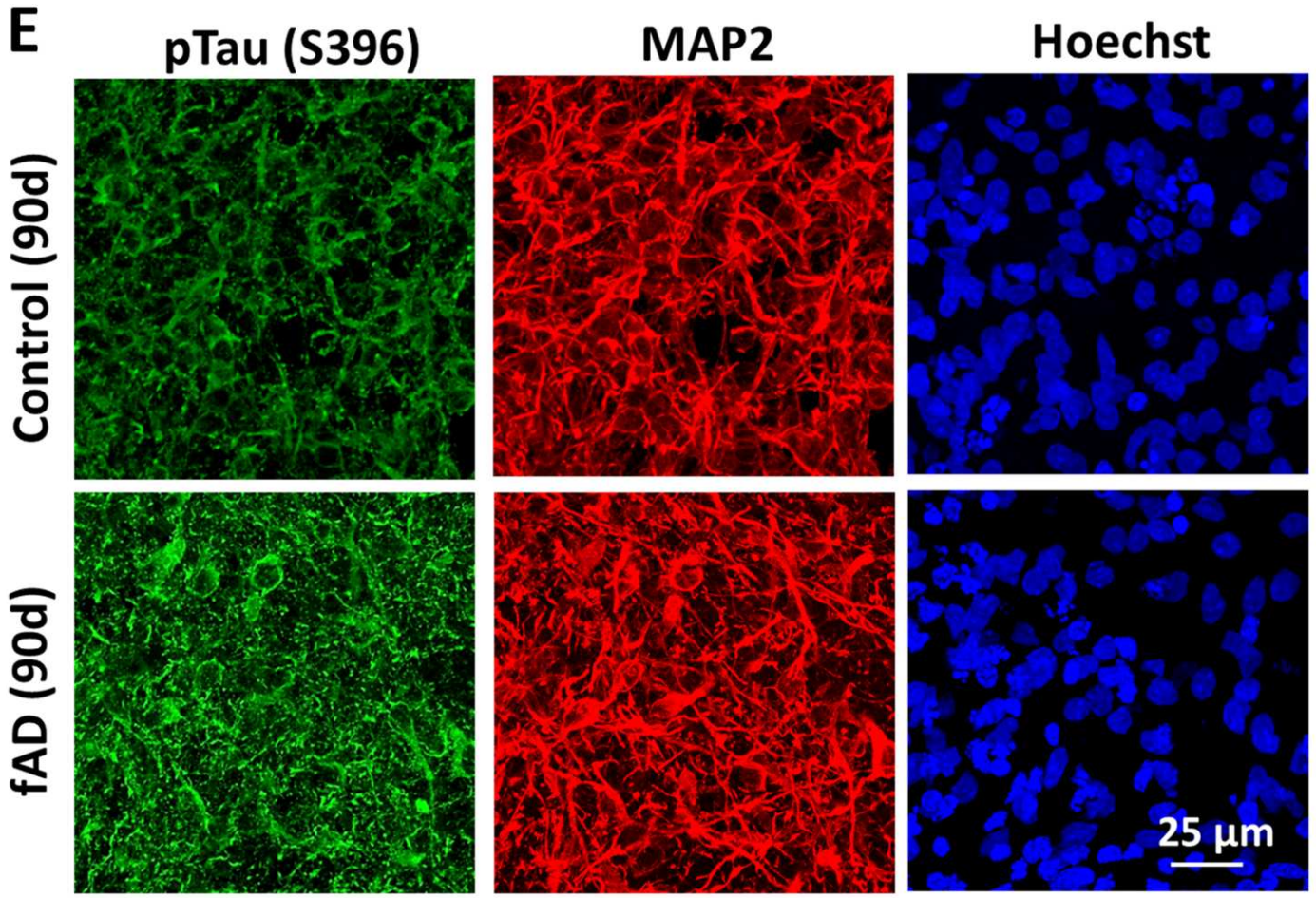
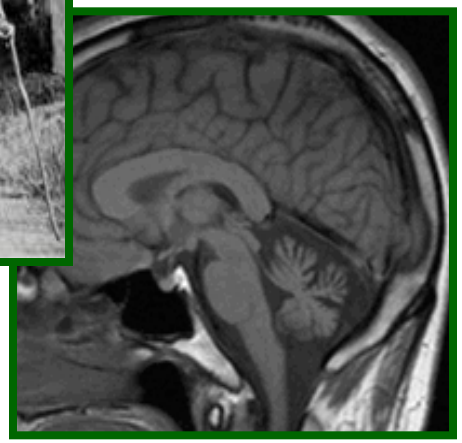


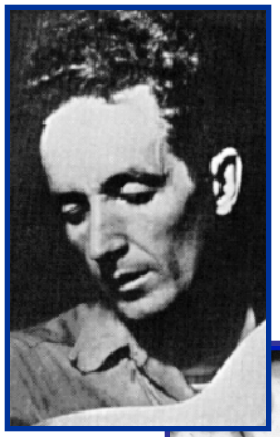
Lecture Overview

- Alzheimer's disease
- Nucleotide expansion (neurodegenerative) disorders and their key pathogenic mechanisms
- Relatively new disease pathogenesis
  - spreading of mutant disease-causing aggregates

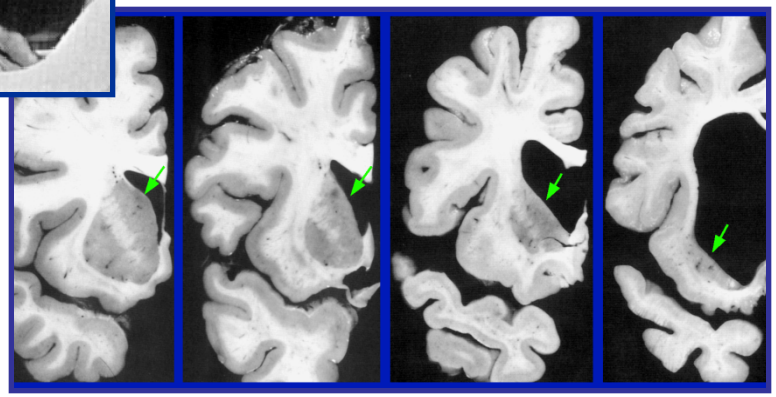
Polyglutamine diseases

SCAs

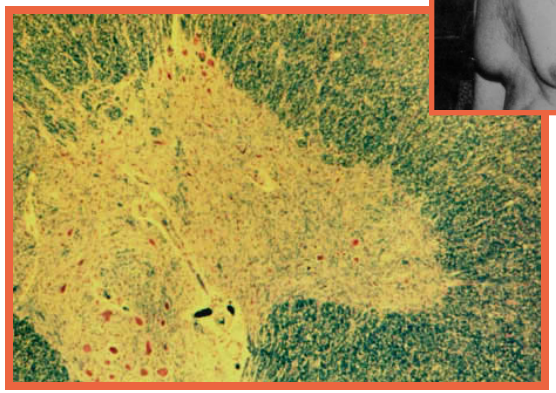




HD

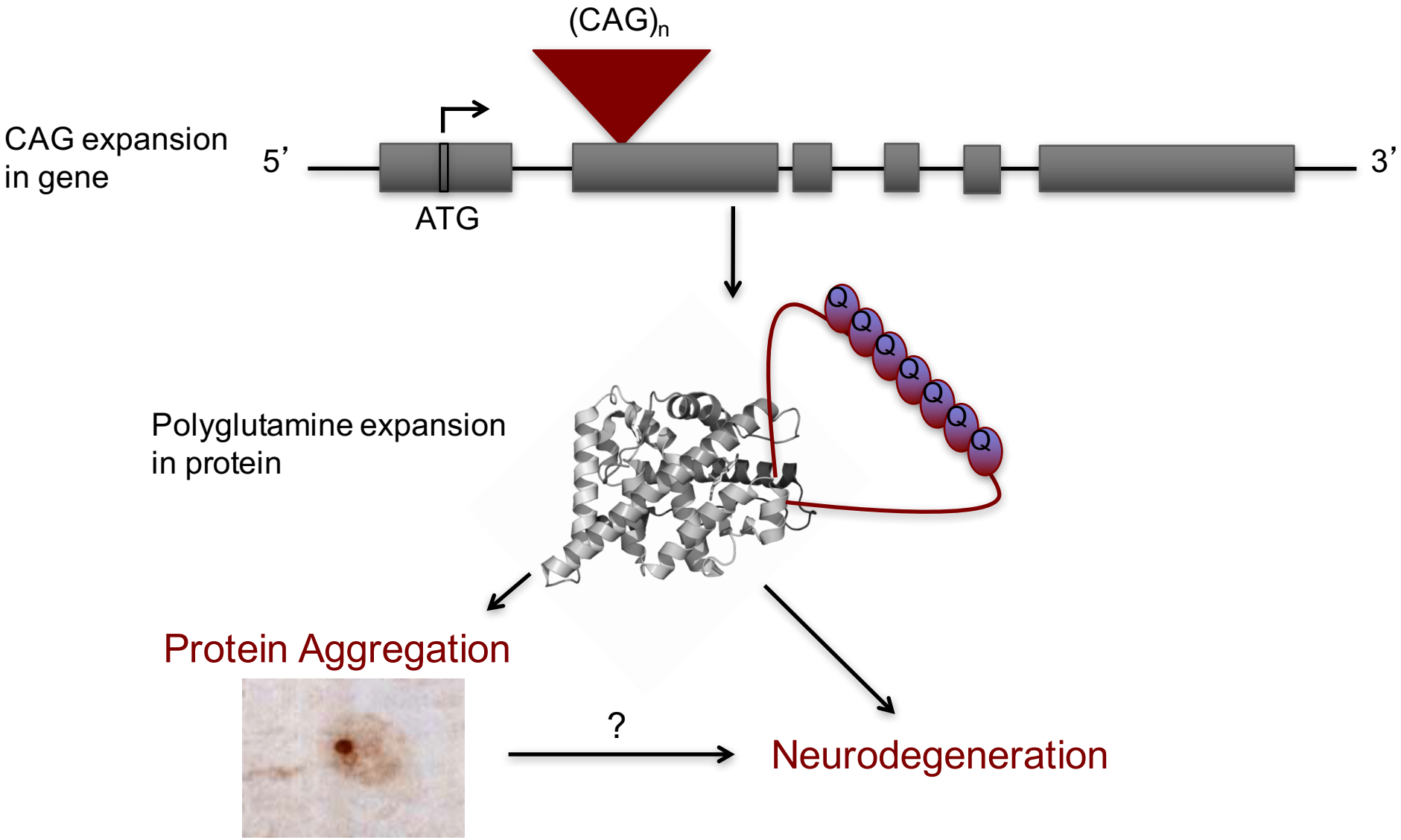


SBMA

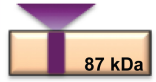
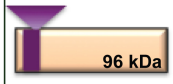


Huntington disease
Spinocerebellar ataxias
Spinobulbar muscular atrophy

Abnormal expansion of CAG repeats causes several human diseases

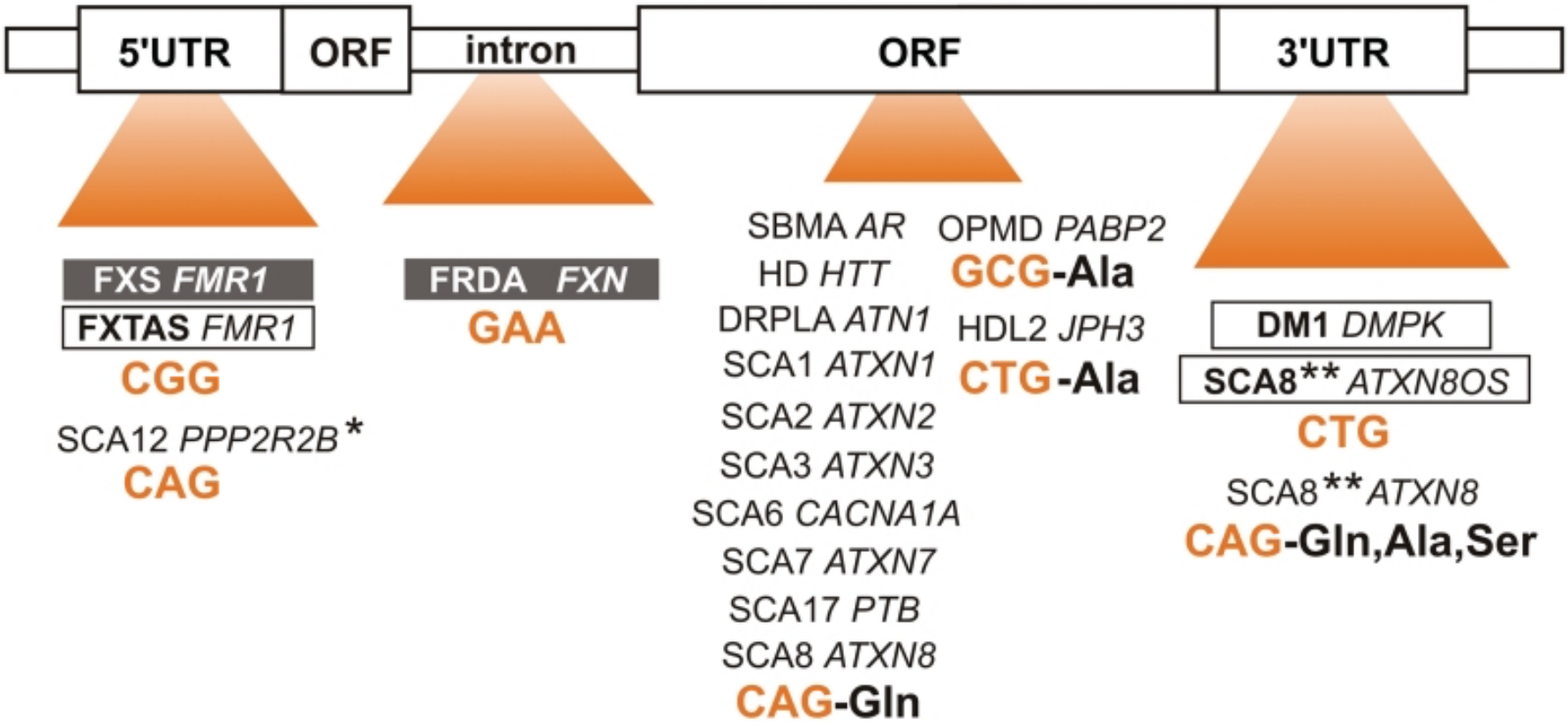


CAG/polyQ expansion confers neurotoxic property that increases with longer repeats

Disease Name	Protein	Wildtype Q length	Expanded Q length	Affected tissues
Huntington's Disease (HD)	Huntingtin (HTT) 	6-35	36-121	Striatum (caudate nucleus, putamen), globus pallidus, Cerebral cortex
Spinal and Bulbar Muscular Atrophy (SBMA)	Androgen Receptor (AR) 	6-36	38-62	Motorneurons of the Anterior Horn and Bulbar Regions, Dorsal Root Ganglia, Skeletal Muscle
Dentatorubral-pallidoluysian Atrophy (DRPLA)	Atrophin-1 (ATN1) 	3-38	49-88	Cerebellum (dentate nucleus), cerebral cortex, globus pallidus, basal ganglia
Spinocerebellar Ataxia Type 1 (SCA1)	ATAXIN-1 (ATXN1) 	6-34	39-83	Cerebellum (Purkinje cells and dentate nucleus), inferior olive, pons, anterior horn cells and pyramidal tracts
SCA2	ATAXIN-2 (ATXN2) 	15-32	32-200	Cerebellum (Purkinje cells), inferior olive, pons, substantia nigra, frontotemporal lobes
Machado-Joseph Disease (MJD)/SCA3	ATAXIN-3 (ATXN3) 	12-40	61-86	Globus pallidus, cerebellum (molecular layer), pons, substantia nigra, anterior horn cells
SCA6	α1A subunit of the voltage-gated Ca^{2+} channel CACNA1A 	4-19	21-33	Cerebellum (Purkinje cells, molecular and granular layers), inferior olive
SCA7	ATAXIN-7 (ATXN7) 	4-35	37-306	Cerebellum (Purkinje cells, molecular and granular layers), pons, inferior olive, visual cortex
SCA17	TATA-Binding Protein (TBP) 	25-43	45-63	Cerebelllum (Purkinje cells), inferior olive

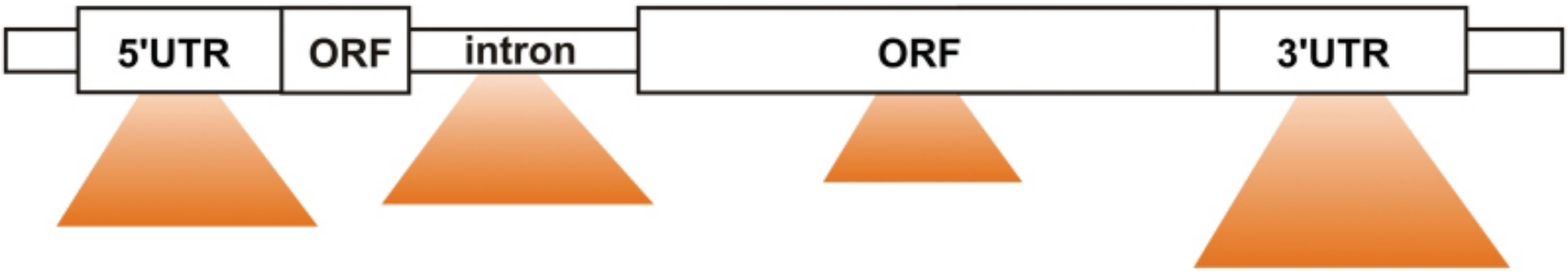
Todd and Lim, *Mol. Cells* 2013

Expanded nucleotide repeats are located in different parts of transcripts

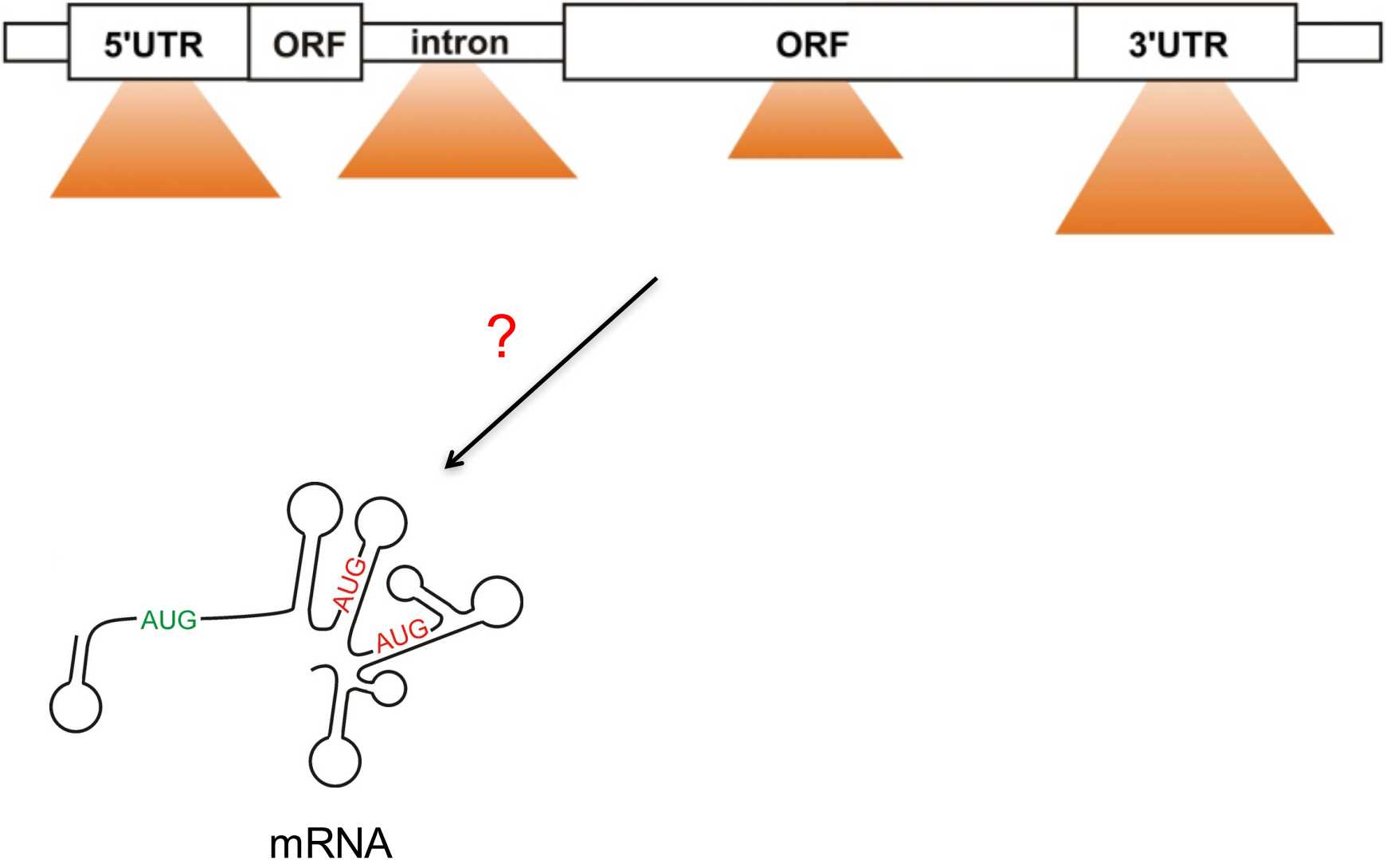


Krzyzosiak et al., *Nucleic Acids Res.* 2011

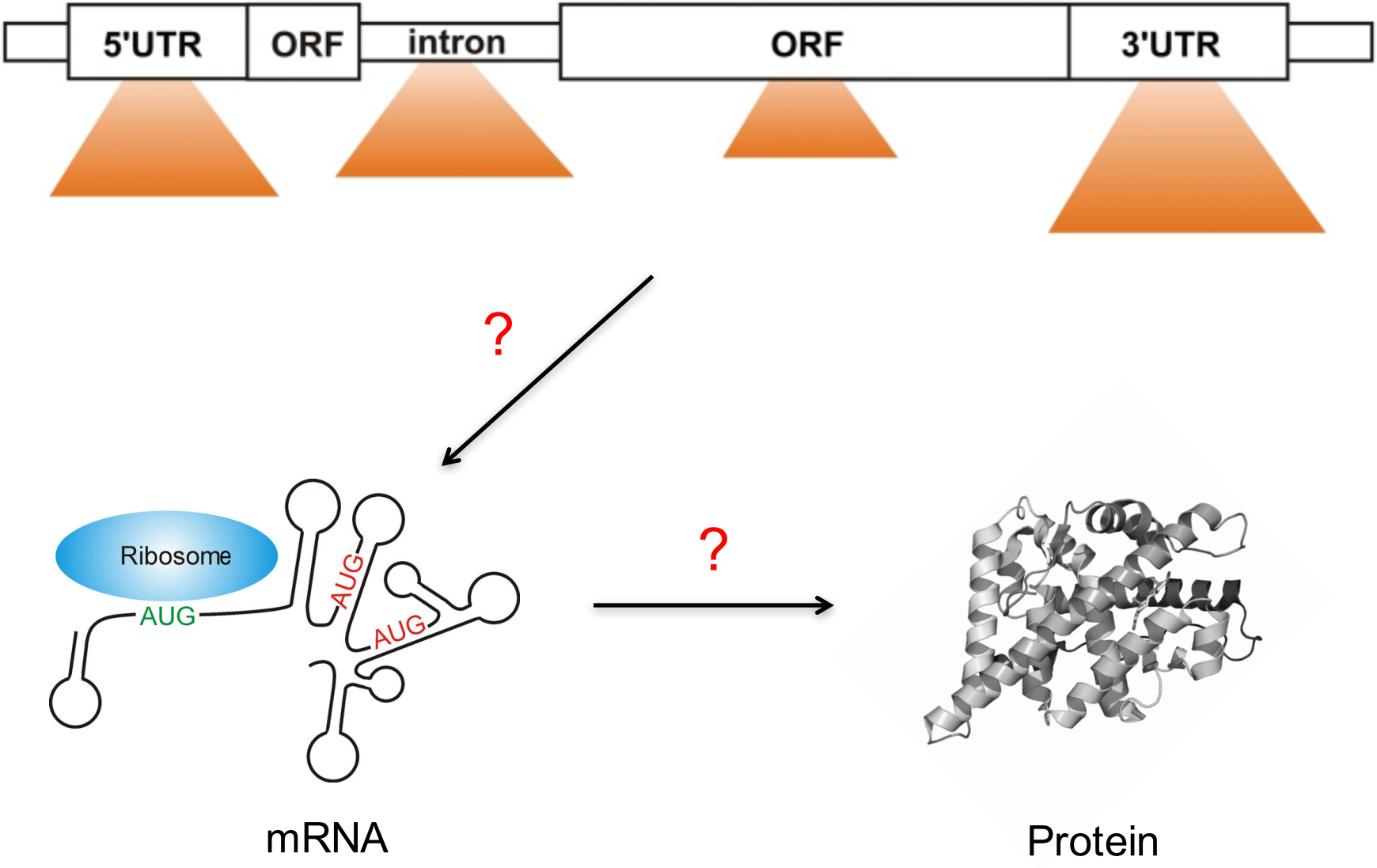
Nucleotide repeat expansion disorders



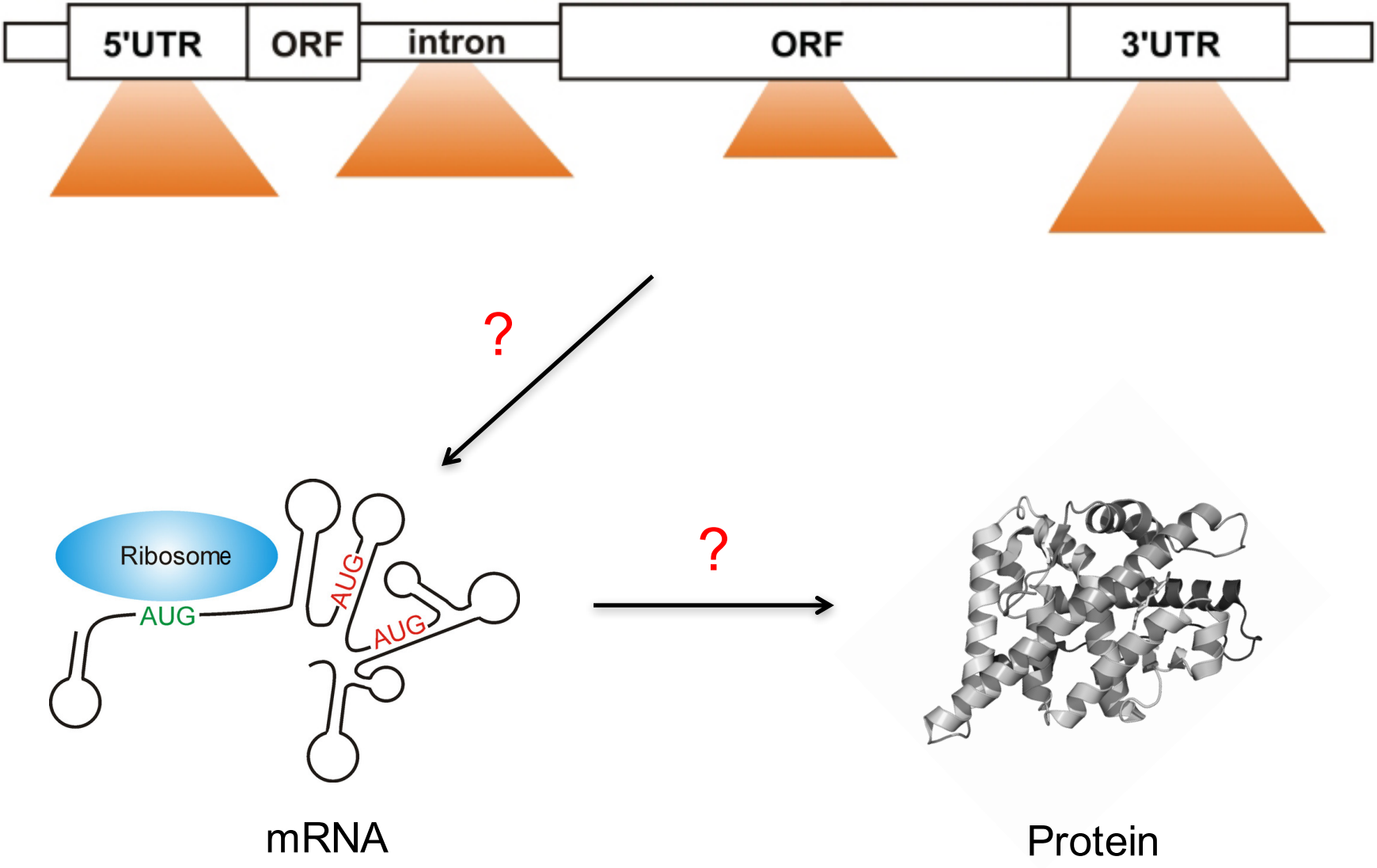
Nucleotide repeat expansion disorders



Nucleotide repeat expansion disorders

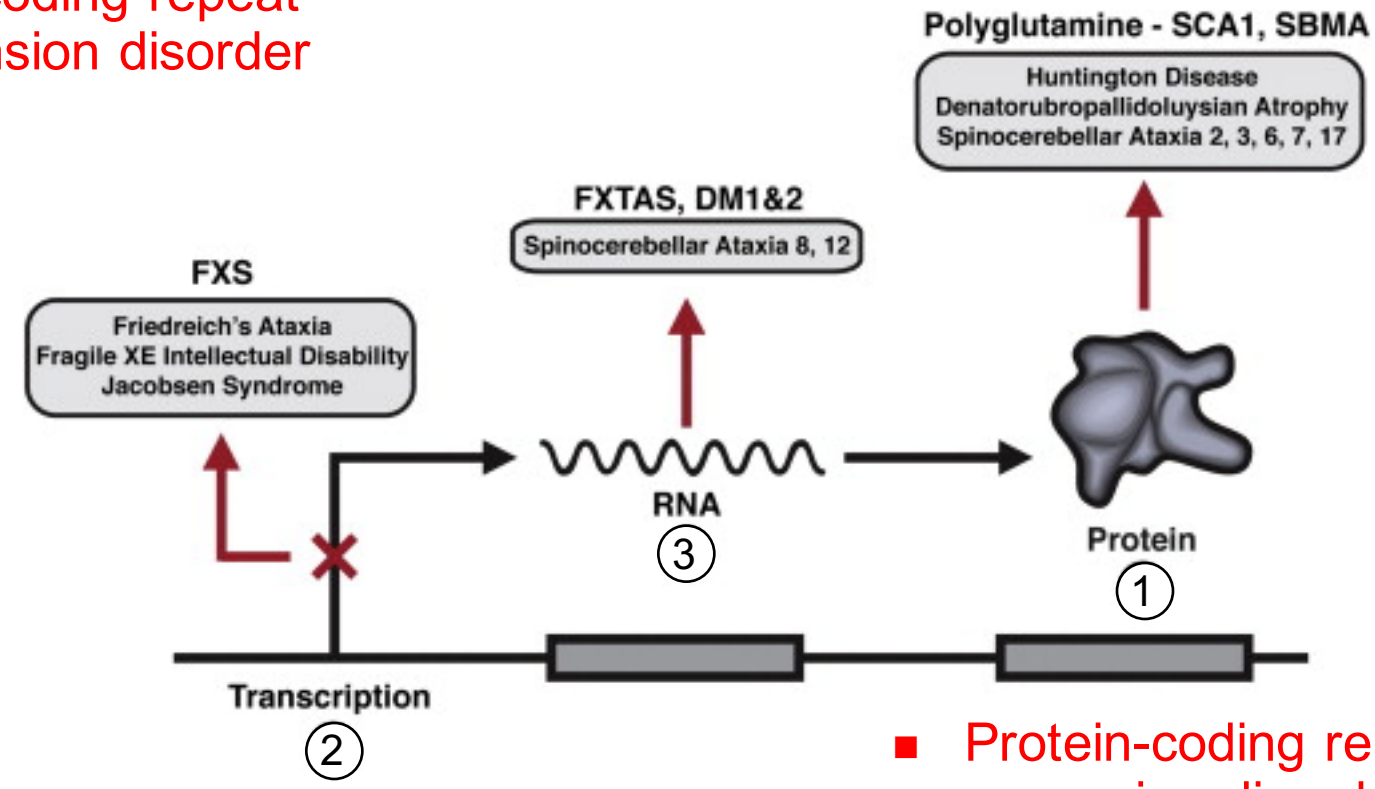


Where does toxicity come from?



Three pathogenic mechanisms for nucleotide repeat expansion disorders

- Non-coding repeat expansion disorder



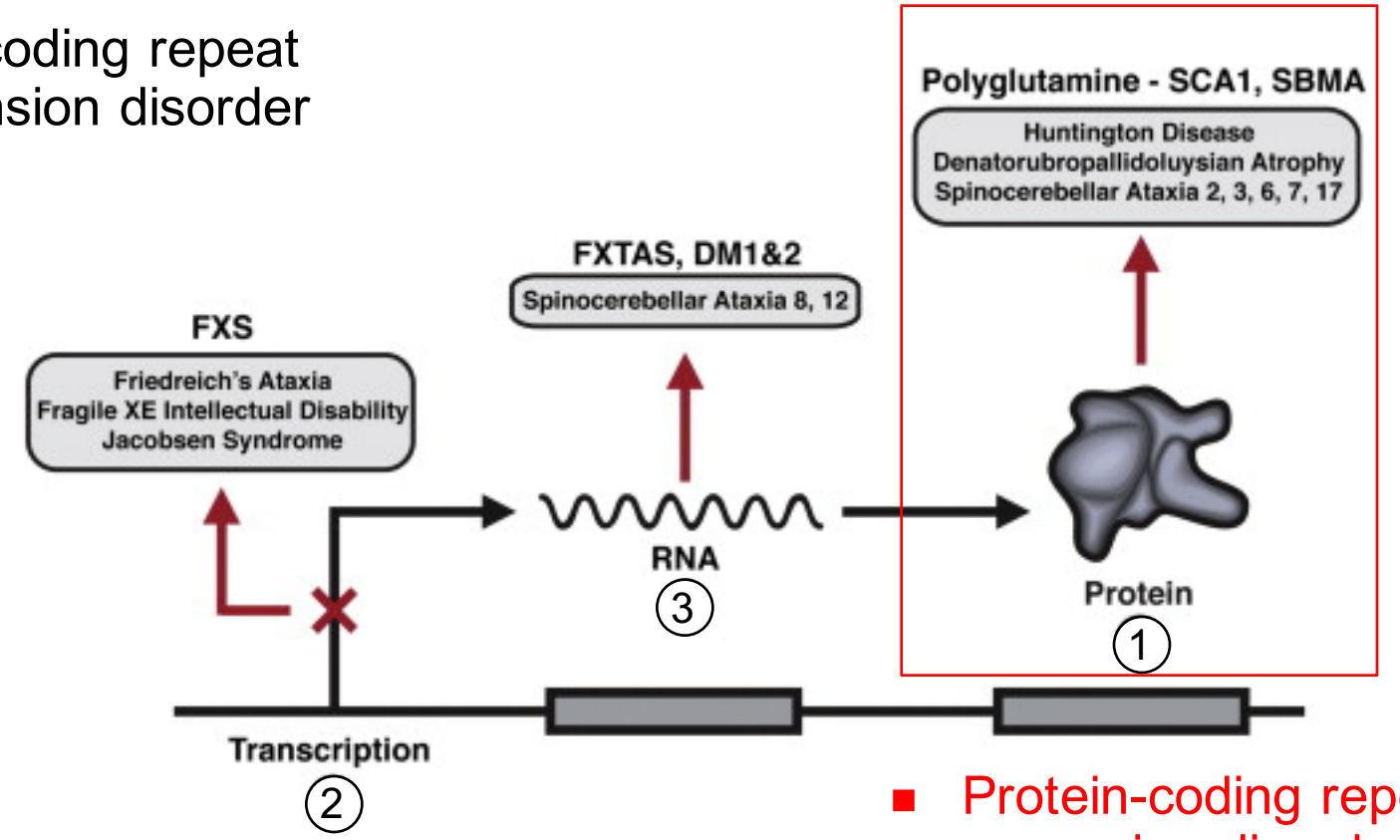
- Protein-coding repeat expansion disorder

1. Protein gain-of-function
2. Loss of gene function
3. RNA gain-of-function

Nelson et al., *Neuron* 2013

Three pathogenic mechanisms for nucleotide repeat expansion disorders

- Non-coding repeat expansion disorder



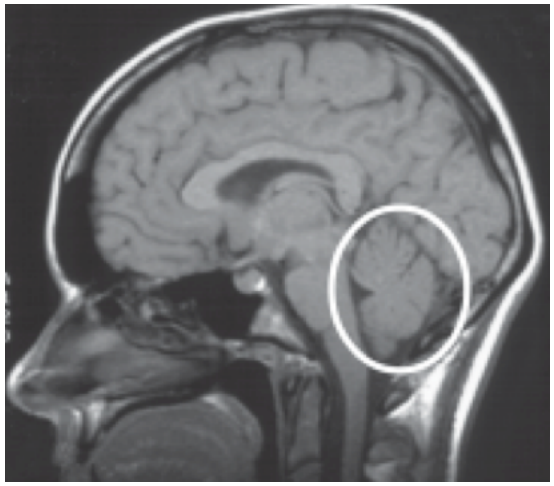
- Protein-coding repeat expansion disorder

1. Protein gain-of-function
2. Loss of gene function
3. RNA gain-of-function

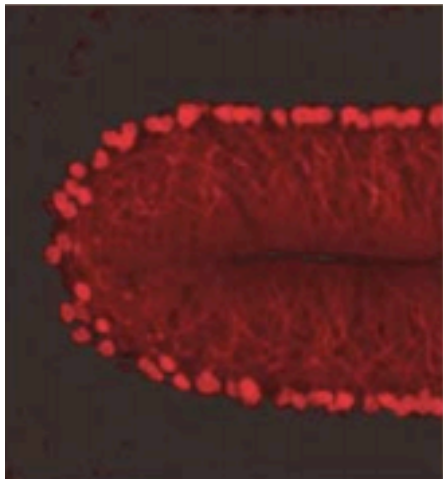
Nelson et al., *Neuron* 2013

Polyglutamine expansion causes cerebellar neurodegeneration in SCA1

Healthy Human

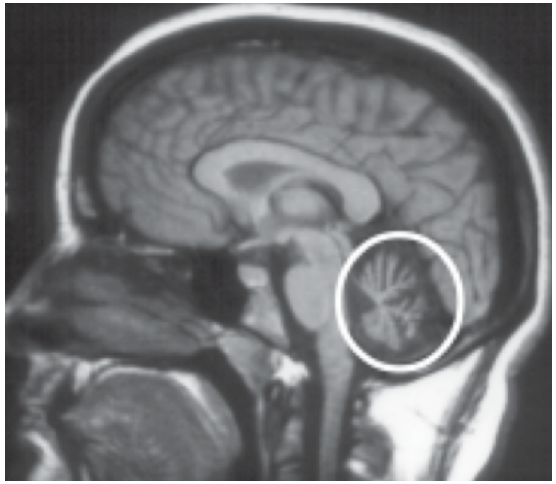


Mouse Wildtype

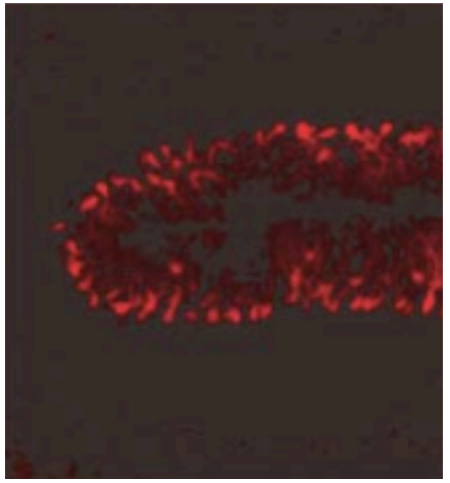


ATXN1 [30Q]

Human SCA1



Mouse SCA1

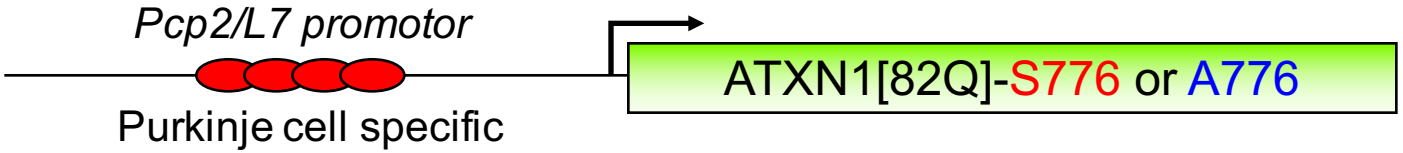


ATXN1 [82Q]

- PolyQ expansion in ATXN1 is necessary but not sufficient to cause ataxia and Purkinje cell degeneration.

Serine 776 is crucial for toxicity of polyglutamine-expanded mutant ATXN1

- SCA1 Transgenic mice

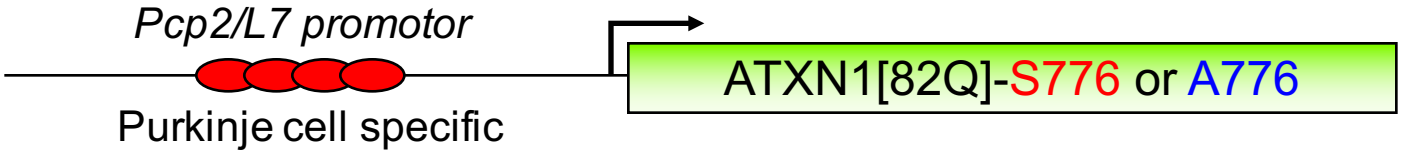


Tg-ATXN1[82Q]-S776

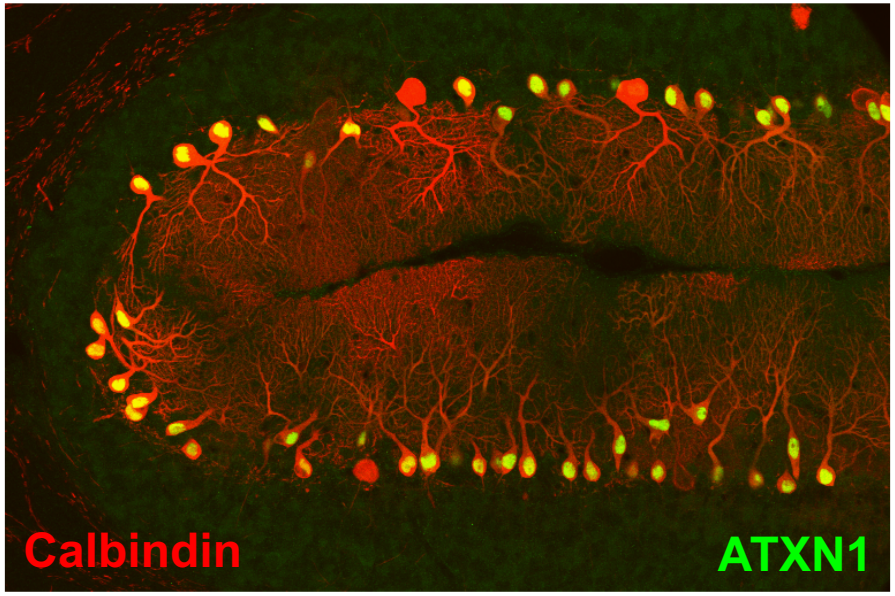
Tg-ATXN1[82Q]-A776

Serine 776 is crucial for toxicity of polyglutamine-expanded mutant ATXN1

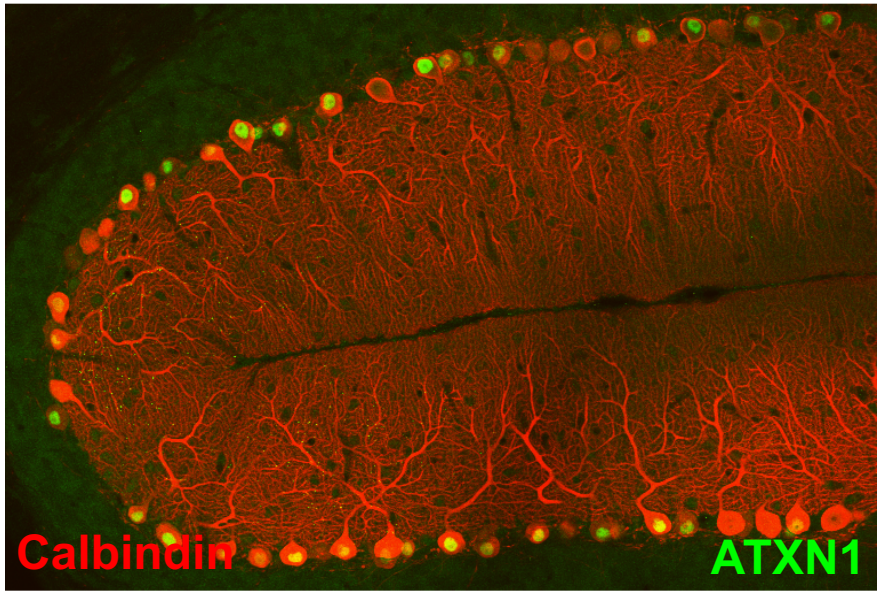
- SCA1 Transgenic mice



Tg-ATXN1[82Q]-S776



Tg-ATXN1[82Q]-A776



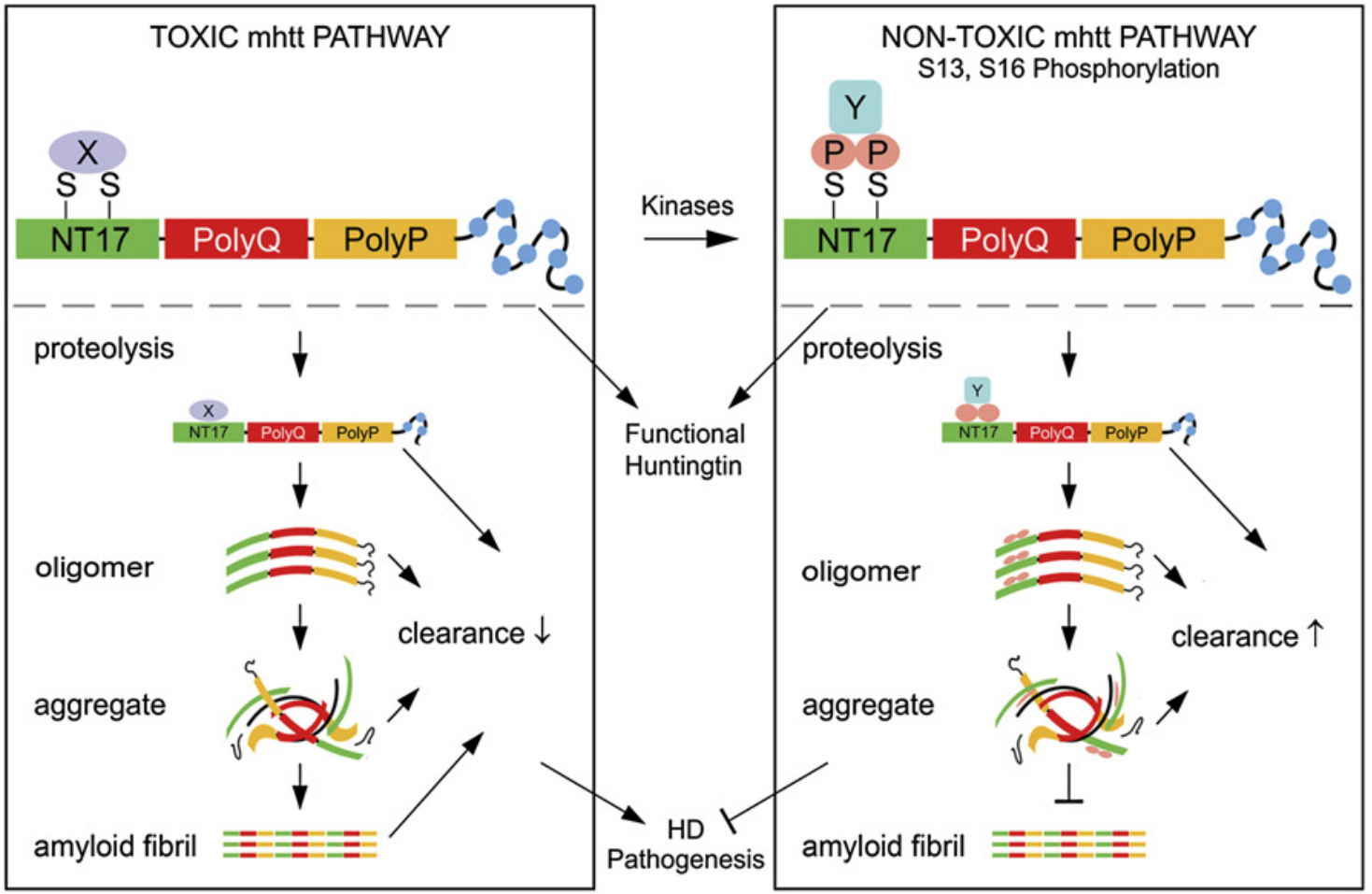
Emammian et al., Neuron, 2003

Neuron
Article

Serines 13 and 16 Are Critical Determinants of Full-Length Human Mutant Huntingtin Induced Disease Pathogenesis in HD Mice

Xiaofeng Gu,[1,2,3] Erin R. Greiner,[1,4] Rakesh Mishra,[5] Ravindra Kodali,[5] Alex Osmand,[6] Steven Finkbeiner,[7] Joan S. Steffan,[8] Leslie Michels Thompson,[8,9,10] Ronald Wetzel,[5] and X. William Yang[1,2,3,*]

[1]Center for Neurobehavioral Genetics, The Jane and Terry Semel Institute for Neuroscience and Human Behavior
[2]Department of Psychiatry and Biobehavioral Sciences
[3]Brain Research Institute
David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA 90095, USA
[4]Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA 90095, USA
[5]Department of Structural Biology and Pittsburgh Institute for Neurodegenerative Diseases, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA
[6]Department of Medicine, University of Tennessee Graduate School of Medicine, Knoxville, TN 37920, USA
[7]Gladstone Institute of Neurological Disease, Taube-Koret Center for Huntington's Disease Research, Departments of Neurology and Physiology, University of California, San Francisco, San Francisco, CA 94158, USA
[8]Department of Psychiatry and Human Behavior
[9]Department of Neurobiology and Behavior
[10]Department of Biological Chemistry
University of California, Irvine, Irvine, CA 92697, USA
*Correspondence: xwyang@mednet.ucla.edu
DOI 10.1016/j.neuron.2009.11.020



Gu et al., *Neuron,* 2009

Cell

Cleavage at the Caspase-6 Site Is Required for Neuronal Dysfunction and Degeneration Due to Mutant Huntingtin

Rona K. Graham,[1,3] Yu Deng,[1,3] Elizabeth J. Slow,[1,3] Brendan Haigh,[1,3] Nagat Bissada,[1,3] Ge Lu,[1,3] Jacqueline Pearson,[1,3] Jacqueline Shehadeh,[2] Lisa Bertram,[1,3] Zoe Murphy,[1,3] Simon C. Warby,[1,3] Crystal N. Doty,[1,3] Sophie Roy,[4] Cheryl L. Wellington,[3] Blair R. Leavitt,[1,3] Lynn A. Raymond,[2] Donald W. Nicholson,[4] and Michael R. Hayden[1,3,*]

[1] Centre for Molecular Medicine and Therapeutics, Department of Medical Genetics

[2] Department of Psychiatry

[3] Child and Family Research Institute

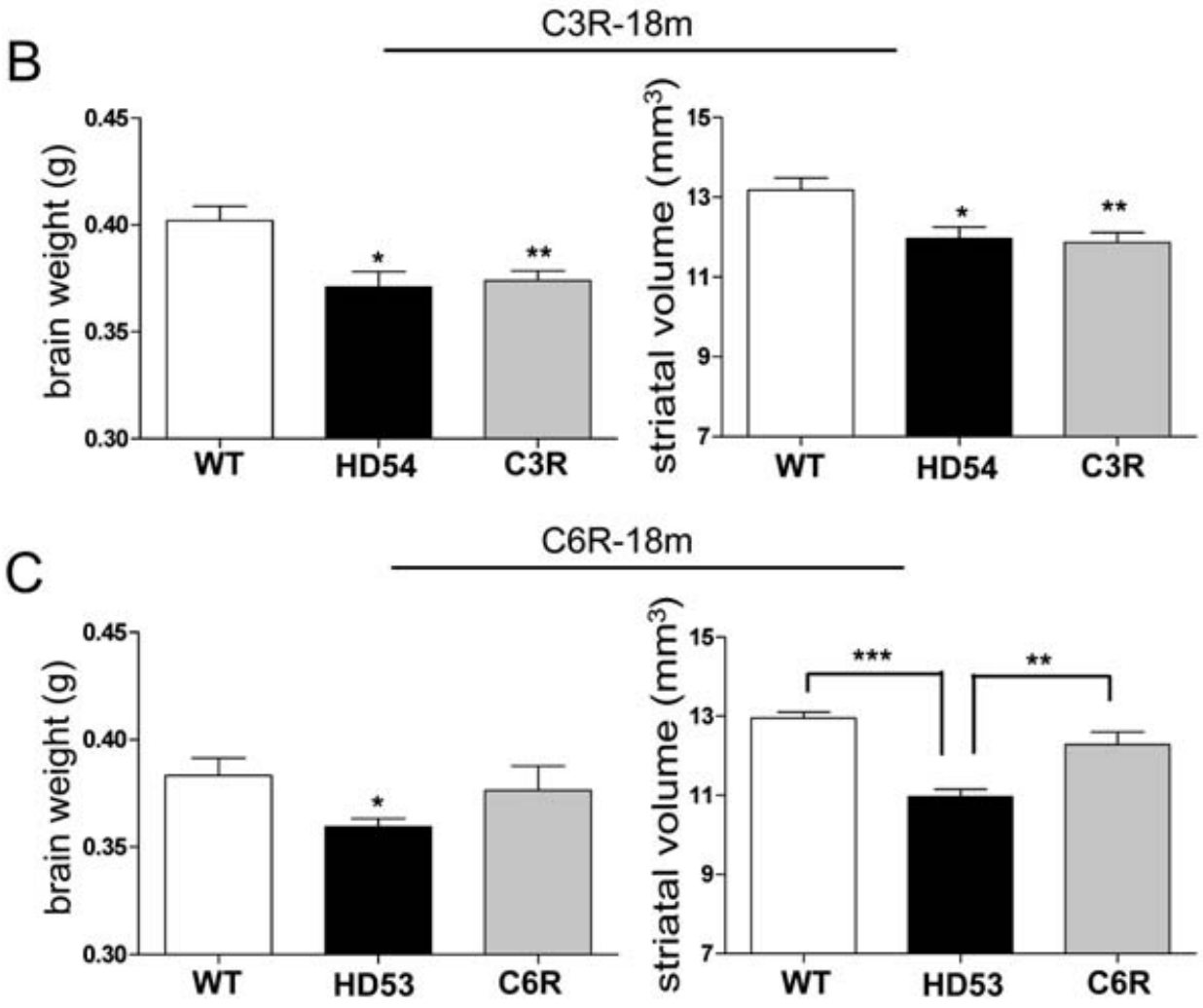
University of British Columbia, Vancouver, BC V5Z 4H4, Canada

[4] Merck Research Laboratories, Whitehouse Station, NJ 08889, USA

*Contact: mrh@cmmt.ubc.ca

DOI 10.1016/j.cell.2006.04.026

- Proteolysis of mutant Htt at the caspase-6 cleavage site is an important event in mediating neuronal dysfunction and neurodegeneration.

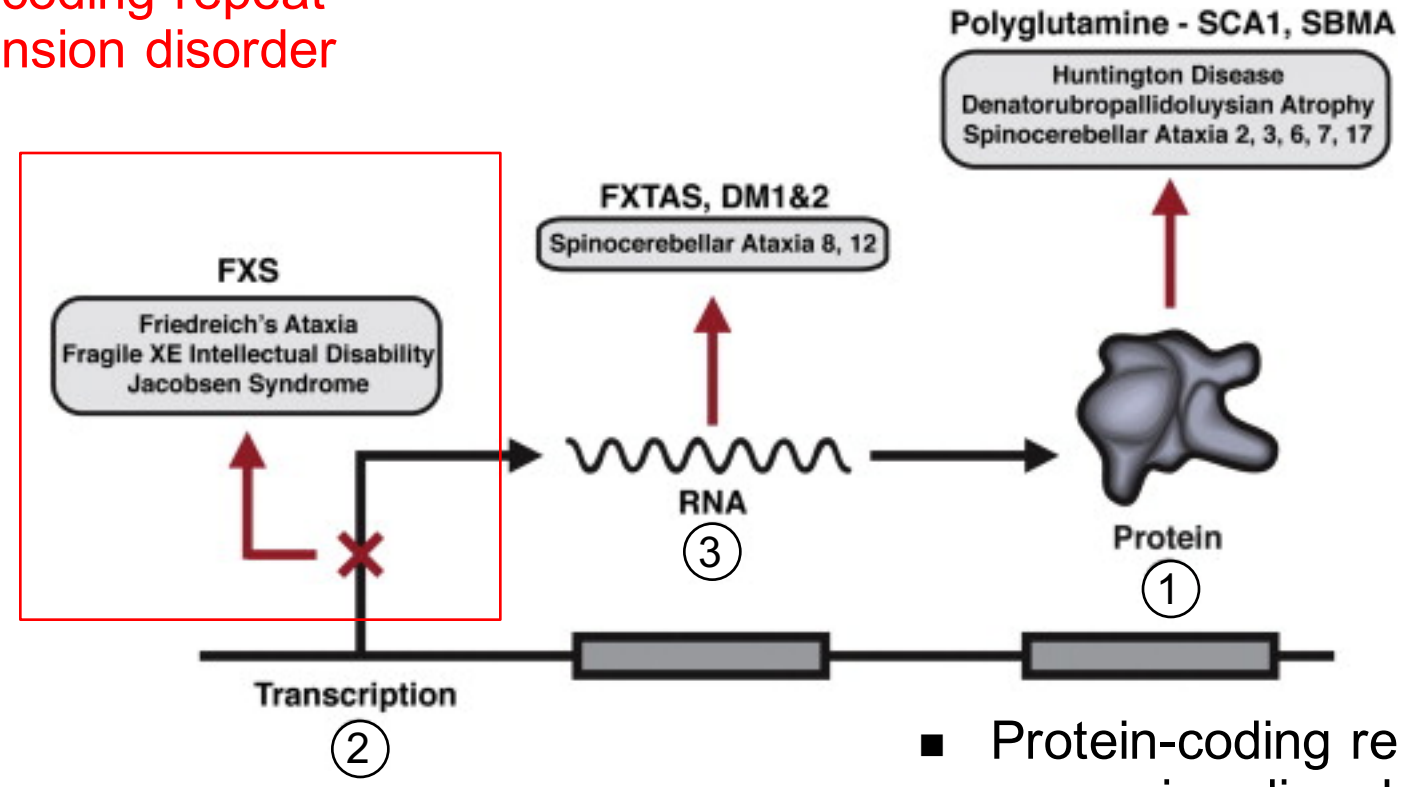


- Inhibition of caspase-6 cleavage, but not caspase-3, of mutant Htt prevents neurodegeneration in mice.

Graham et al., *Cell* 2006

Three pathogenic mechanisms for nucleotide repeat expansion disorders

- Non-coding repeat expansion disorder



- Protein-coding repeat expansion disorder

1. Protein gain-of-function
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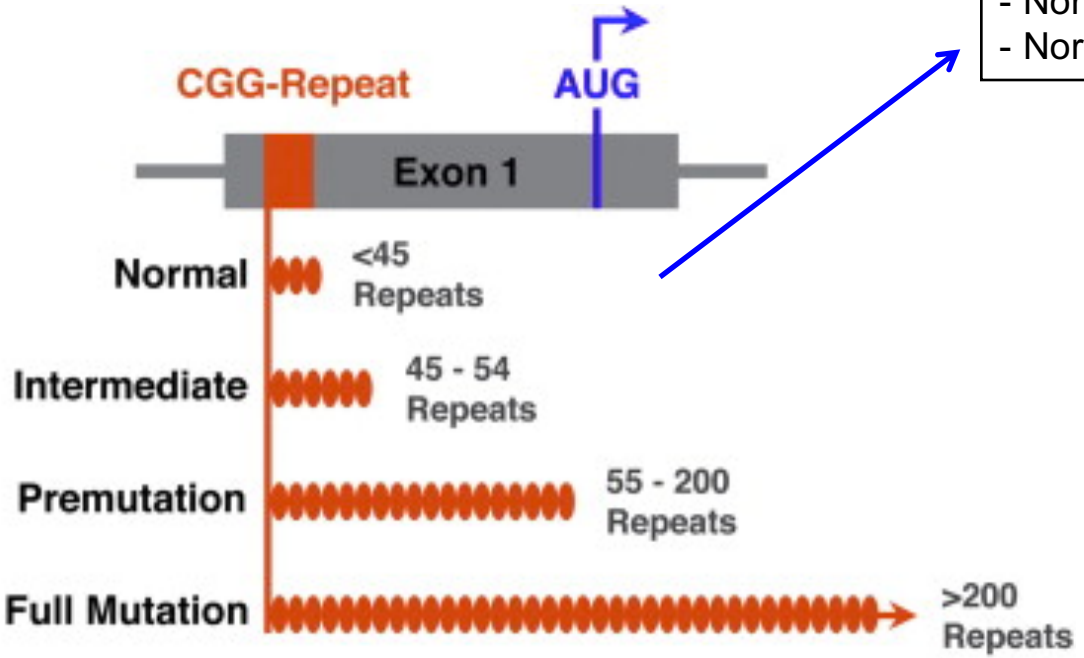
3. RNA gain-of-function

Nelson et al., *Neuron* 2013

- Alleles of *FMR1*

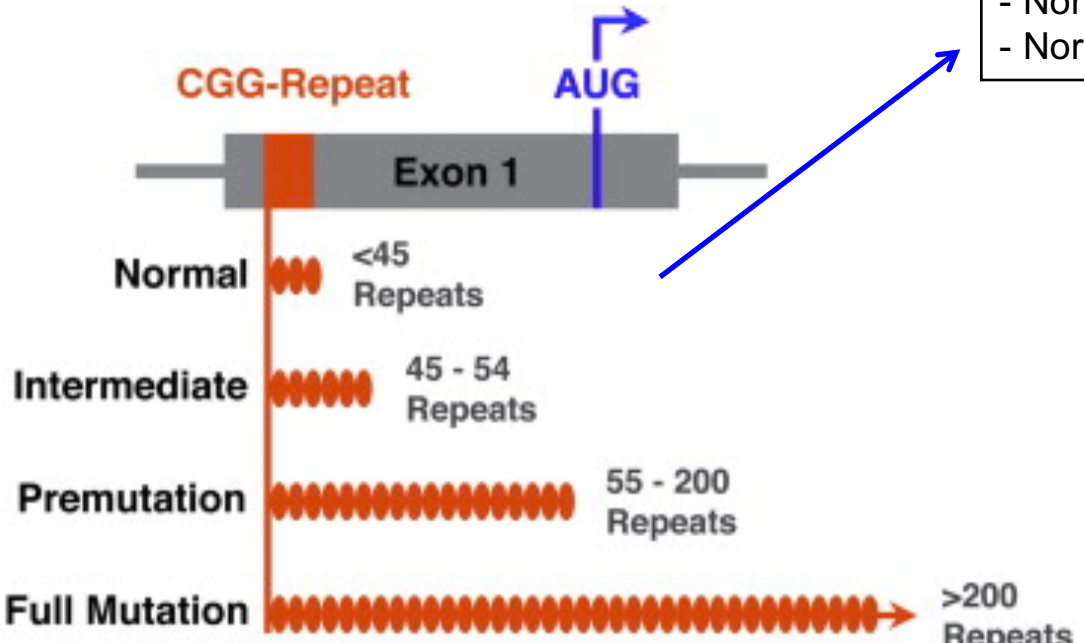


Normal individual
- Normal transcription
- Normal function of FMRP

Nelson et al., *Neuron* 2013

Loss of gene function

- Alleles of *FMR1*



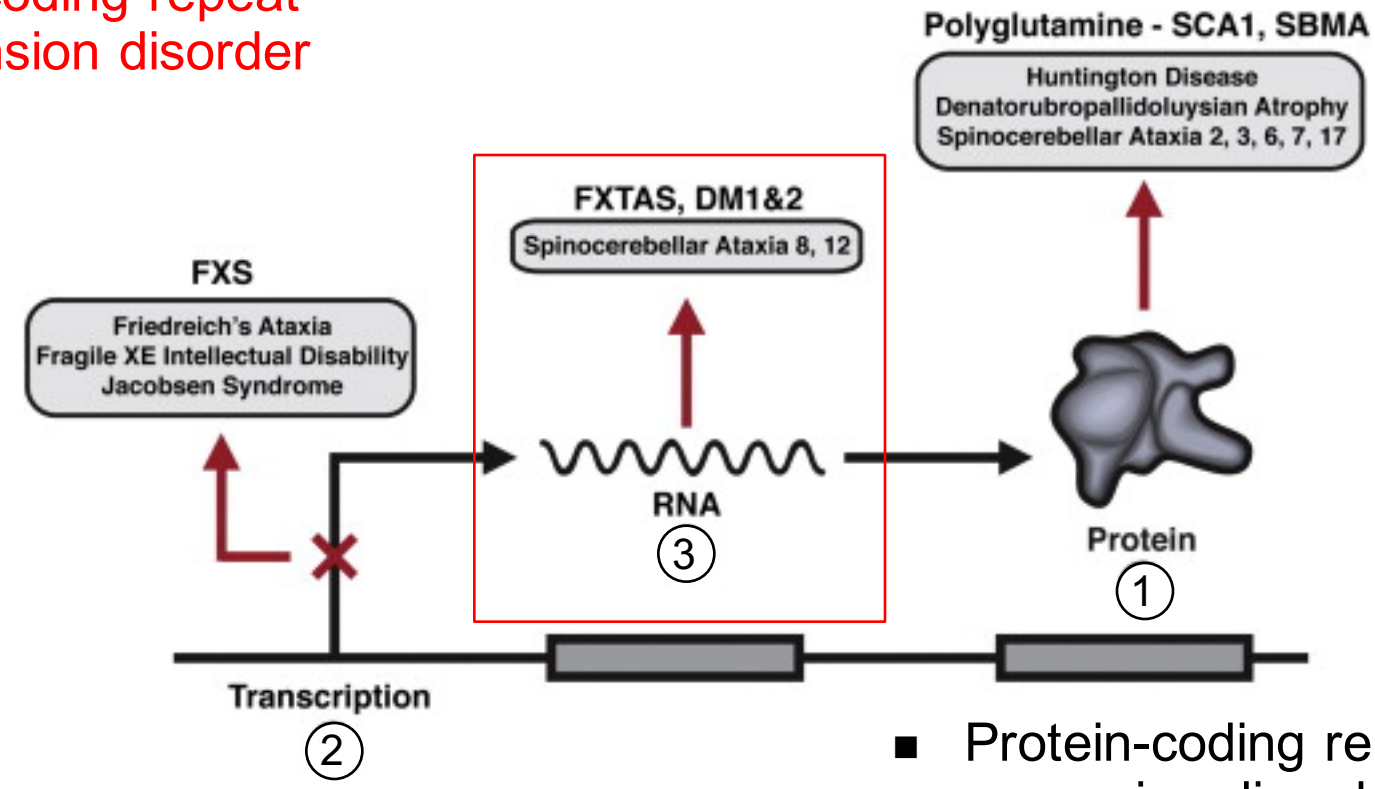
Normal individual
- Normal transcription
- Normal function of FMRP

Fragile X syndrome (FXS)
- Silencing *FMR1* transcription
- Loss of function of FMRP

Nelson et al., *Neuron* 2013

Three pathogenic mechanisms for nucleotide repeat expansion disorders

- Non-coding repeat expansion disorder



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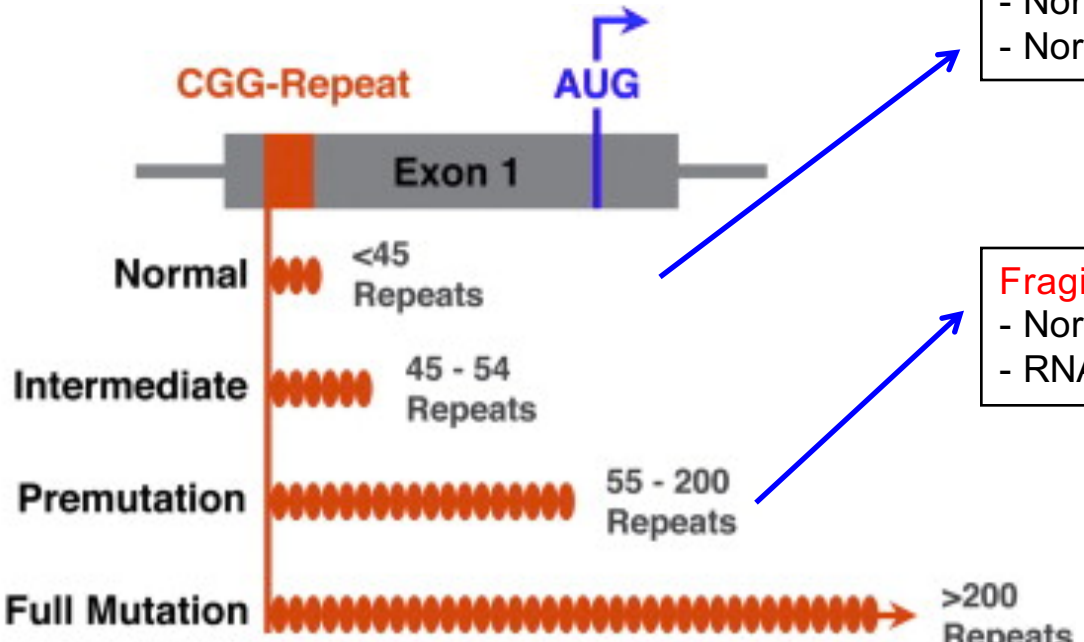
Nelson et al., *Neuron* 2013

RNA gain-of-function

- Alleles of *FMR1*



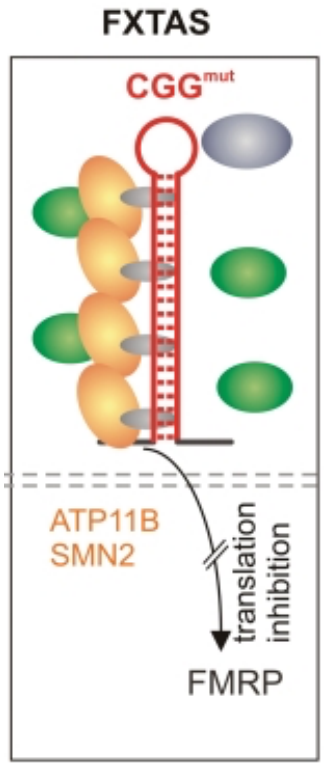
Normal individual
- Normal transcription
- Normal function of FMRP

Fragile X tremor-ataxia syndrome (FXTAS)
- Normal transcription
- RNA gain-of-function

Fragile X syndrome (FXS)
- Silencing *FMR1* transcription
- Loss of gene function

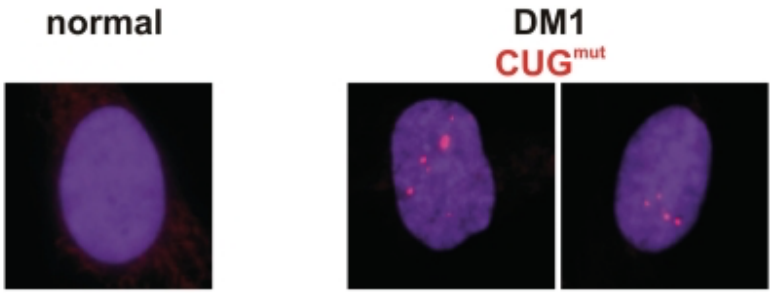
Nelson et al., *Neuron* 2013

Dominant effects of toxic RNA repeats

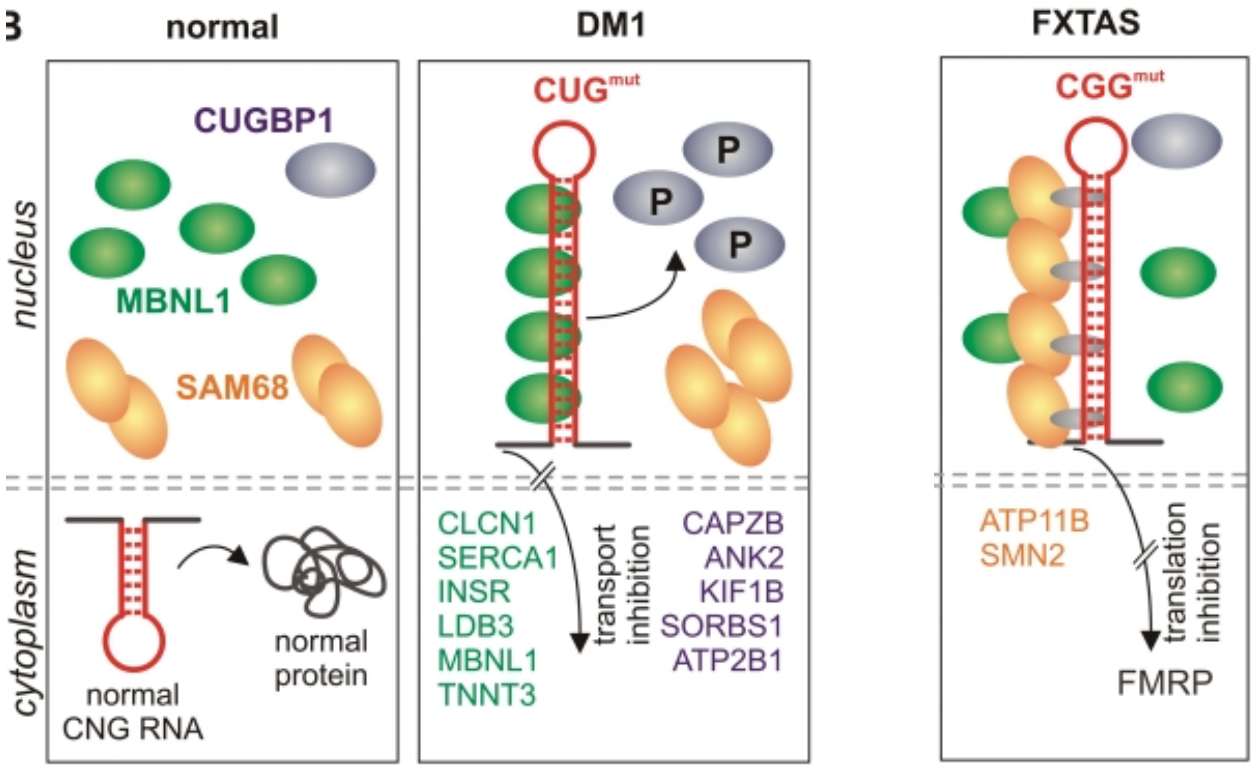


Krzyzosiak et al., *Nucleic Acids Res.* 2011

Dominant effects of toxic RNA repeats



Nuclear RNA foci



Krzyzosiak et al., *Nucleic Acids Res.* 2011

RNA foci and toxicity

- Myotonic dystrophy type 1 (DM1)

  - CTG trinucleotide expansion in the 3'-UTR region of *DMPK1*, which codes for myotonic dystrophy protein kinase.

  - Anticipation: 5-37 repeats (normal) > 38-49 repeats (premutation) > lager than 50 repeats (full mutation)

- Myotonic dystrophy type 2 (DM2)

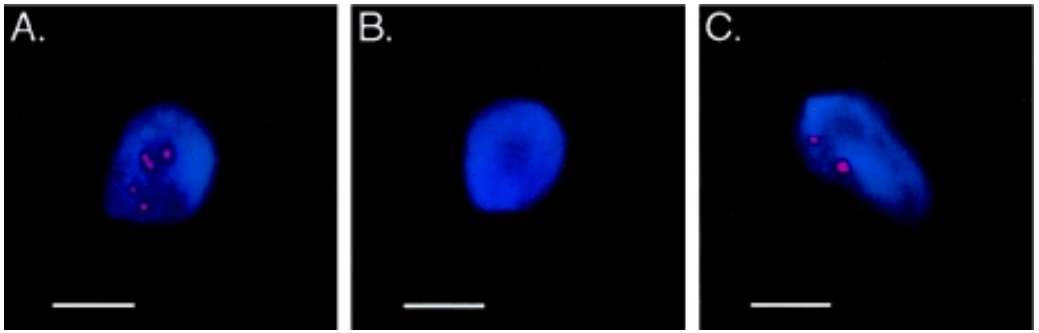
  - CCTG tetranucleotide expansion in the intron 1 of *ZNF9*

  - Mild anticipation: up to 30 repeats (normal) > 75 to over 11,000 repeats (symptomatic)

- RNA foci

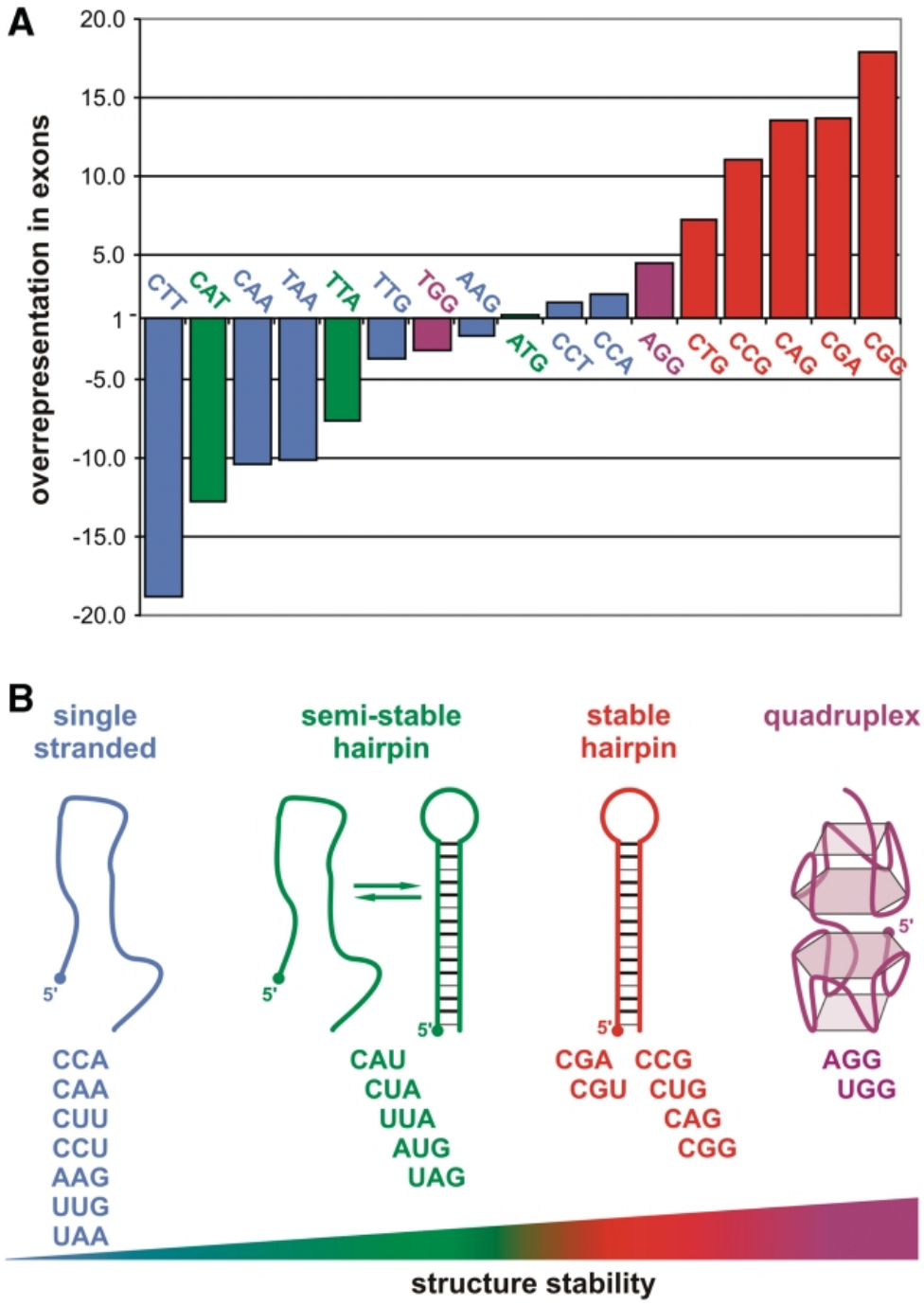
  - lead to the sequestration and altered activity of RNA binding proteins

  - RNA-binding proteins "Muscleblind-like1" and "CUGBP1"



Liquori et al., *Science* 2001

The occurrence of various triplet repeats in the human transcriptome and their RNA structures



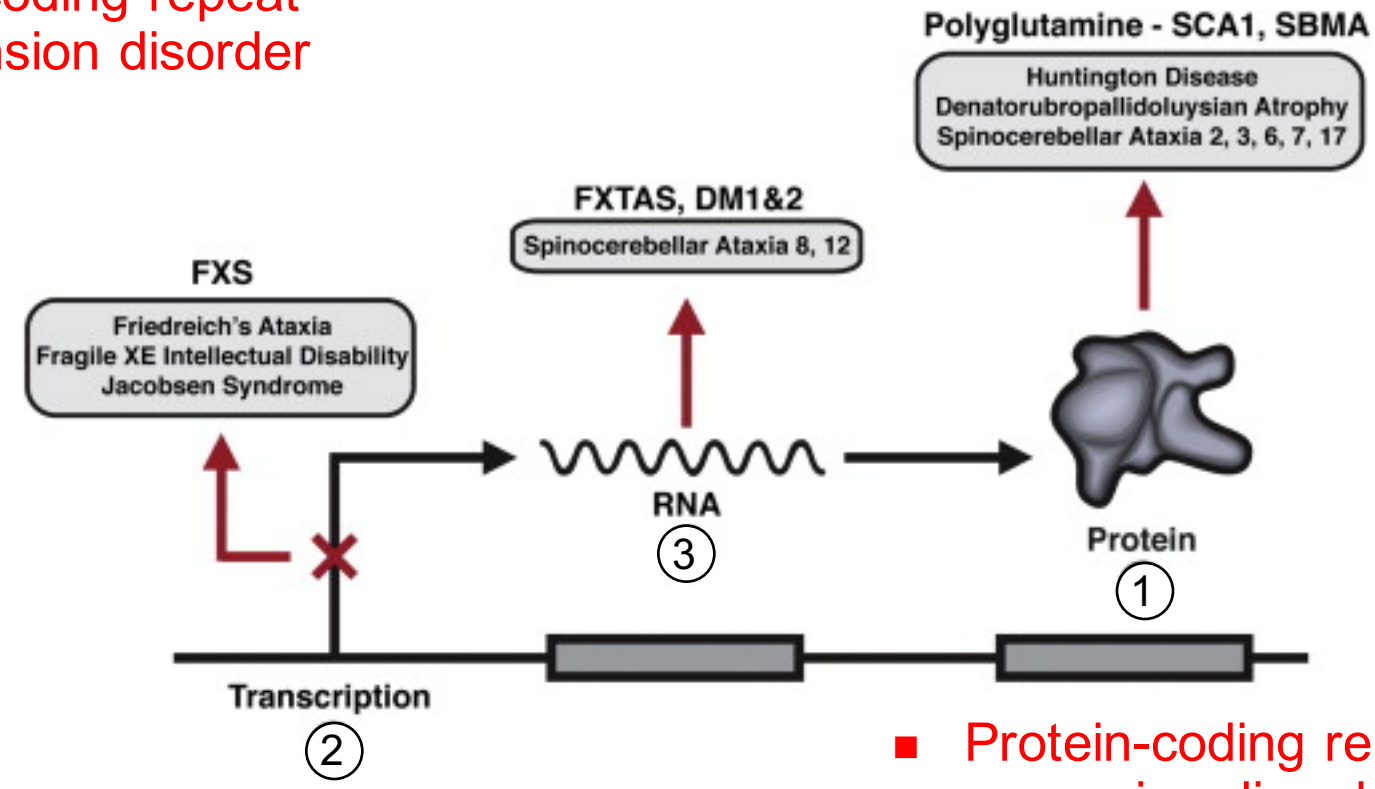
(A) Representation of TNRs composed of at least six repeat units in RefSeq mRNA sequences compared with the whole human genome sequence (17 out of 20 triplets are shown due to lack of CGT, CTA, TAG repeats in exons.

(B) 20 different triplet repeat RNAs belong to four structural classes.

Krzyzosiak et al., *Nucleic Acids Res.* 2011

Three pathogenic mechanisms for nucleotide repeat expansion disorders

- Non-coding repeat expansion disorder



- Protein-coding repeat expansion disorder

1. Protein gain-of-function
2. Loss of gene function
3. RNA gain-of-function

Nelson et al., *Neuron* 2013

Hexanucleotide repeat expansion disorder

- Chromosome 9-linked FTD/ALS

Neuron
Article

Cell PRESS

Neuron 72, 245-256 (2011)

Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of *C9ORF72* Causes Chromosome 9p-Linked FTD and ALS

Mariely DeJesus-Hernandez,[1,10] Ian R. Mackenzie,[2,10,*] Bradley F. Boeve,[3] Adam L. Boxer,[4] Matt Baker,[1] et al.

Neuron
Article

Cell PRESS

Neuron 72, 257-268 (2011)

A Hexanucleotide Repeat Expansion in *C9ORF72* Is the Cause of Chromosome 9p21-Linked ALS-FTD

Alan E. Renton,[1,38] Elisa Majounie,[2,38] Adrian Waite,[3,38] Javier Simón-Sánchez,[4,5,38] Sara Rollinson,[6,38] et al.

Frequency of Chromosome 9p21 Repeat Expansion in FTLD and ALS

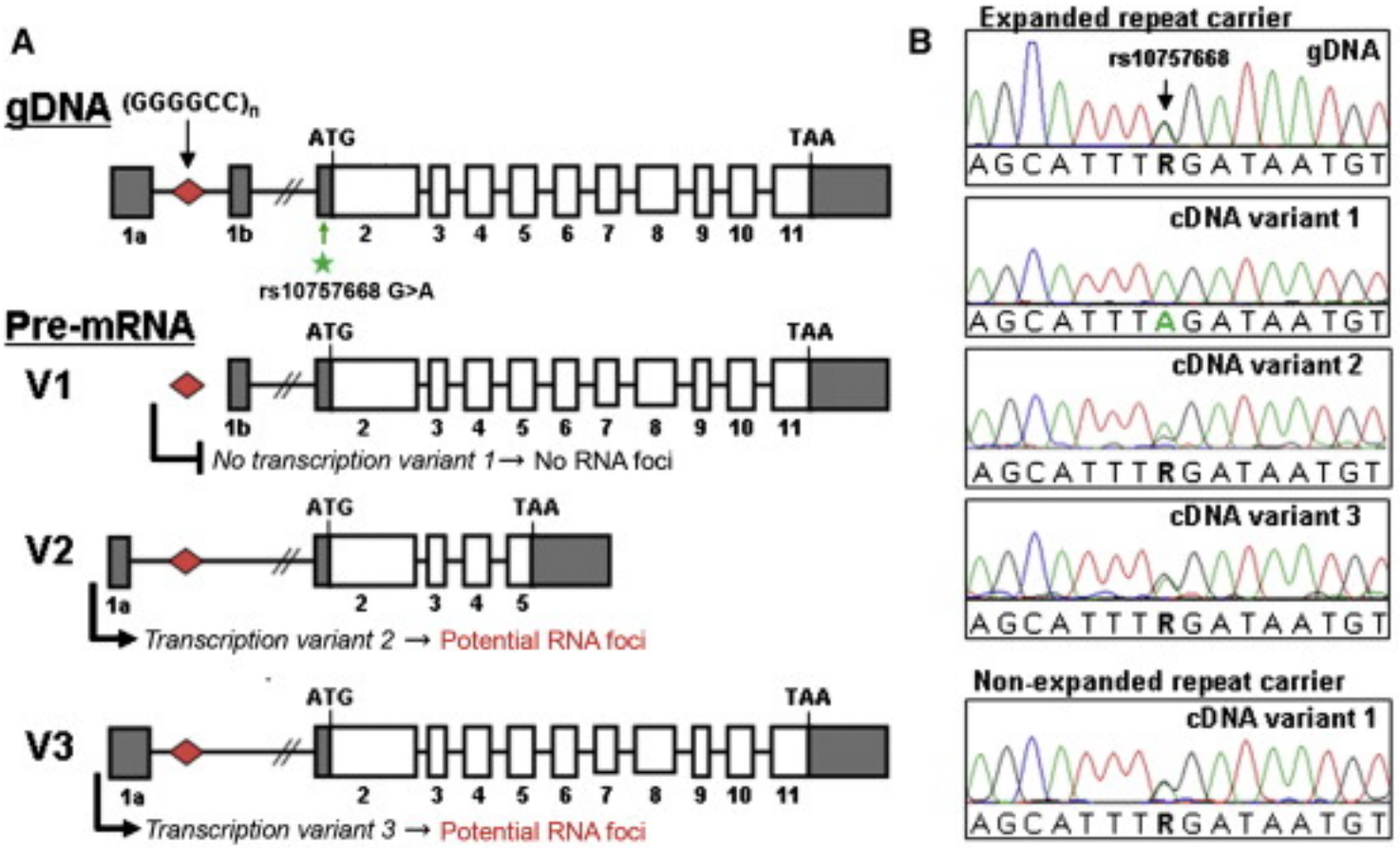
Cohort	n	Number of Mutation Carriers (%)					
		c9FTD/ALS	GRN	MAPT	SOD1	TARDBP	FUS
UBC FTLD-TDP							
Familial	26	16 (61.5)	7 (26.9)	n/a	n/a	n/a	n/a
MCF FTLD-TDP							
Familial	40	9 (22.5)	6 (15.0)	n/a	n/a	n/a	n/a
Sporadica	53	8 (15.1)	8 (15.1)	n/a	n/a	n/a	n/a
MC Clinical FTD							
Familial	171	20 (11.7)	13 (7.6)	12 (6.3)	n/a	n/a	n/a
Sporadic	203	6 (3.0)	6 (3.0)	3 (1.5)	n/a	n/a	n/a
MCF Clinical ALS							
Familial	34	8 (23.5)	n/a	n/a	4 (11.8)	1 (2.9)	1 (2.9)
Sporadic	195	8 (4.1)	n/a	n/a	0 (0.0)	2 (1.0)	3 (1.5)

UBC = University of British Columbia
MCF = Mayo Clinic Florida
MC = Mayo Clinic

- C9ORF72 hexanucleotide expansion is the major cause of sporadic and familial FTD/ALS.
  - at least 8% of sporadic ALS (sALS) and FTD cases
  - more than 40% of familial ALS (FALS) and FTD cases

DeJesus-Hernandez et al., *Neuron* 2011

Effect of expanded hexanucleotide repeat on *C9ORF72* expression

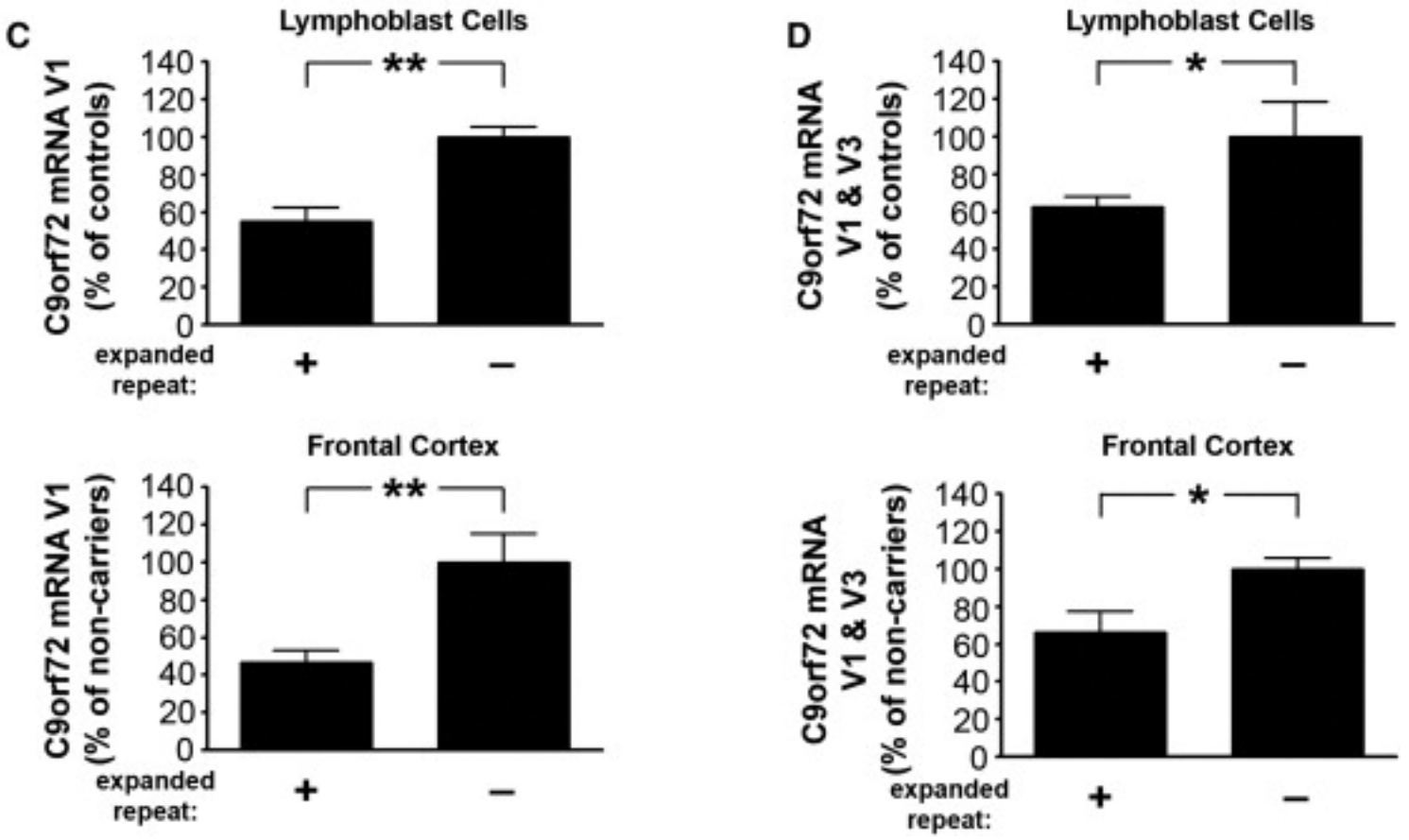


C9ORF72 protein

- Function not well known
- Structurally related to DENN domain proteins, highly conserved GDP-GTP exchanging factors for Rab GTPases.

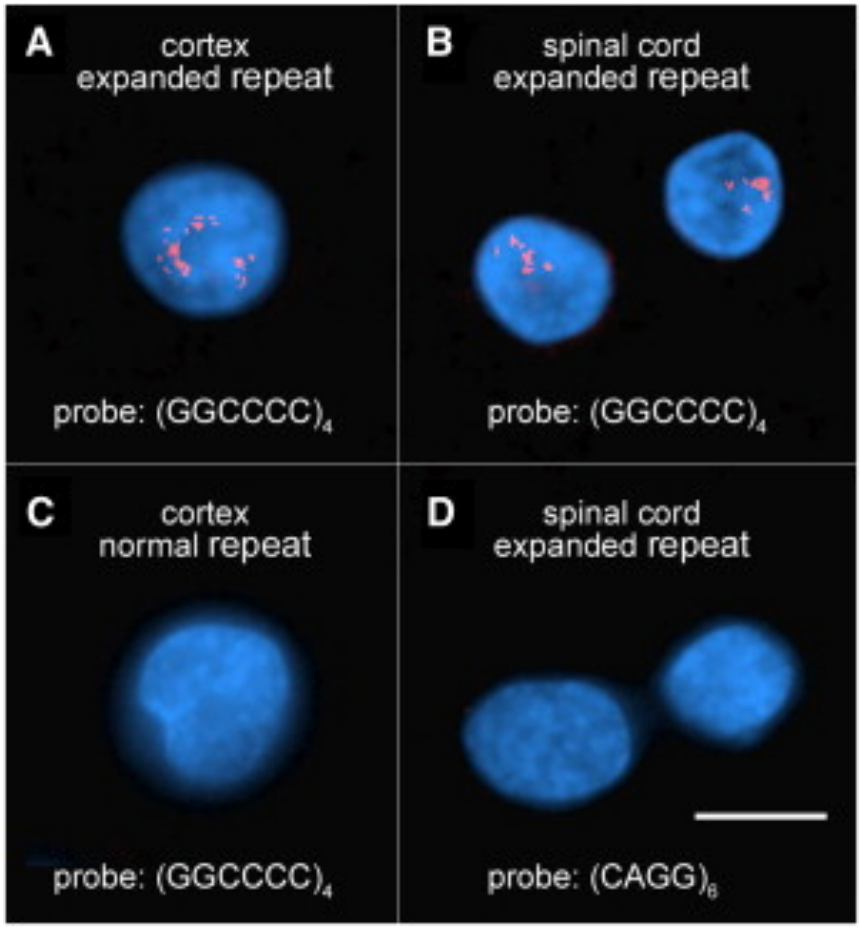
DeJesus-Hernandez et al., *Neuron* 2011

Expansion of hexanucleotide repeats reduces *C9ORF72* mRNA expression, suggesting a potential loss-of-function



DeJesus-Hernandez et al., *Neuron* 2011

Expanded GGGGCC hexanucleotide repeat forms nuclear RNA foci in human brain and spinal cord, suggesting a toxic RNA gain-of-function



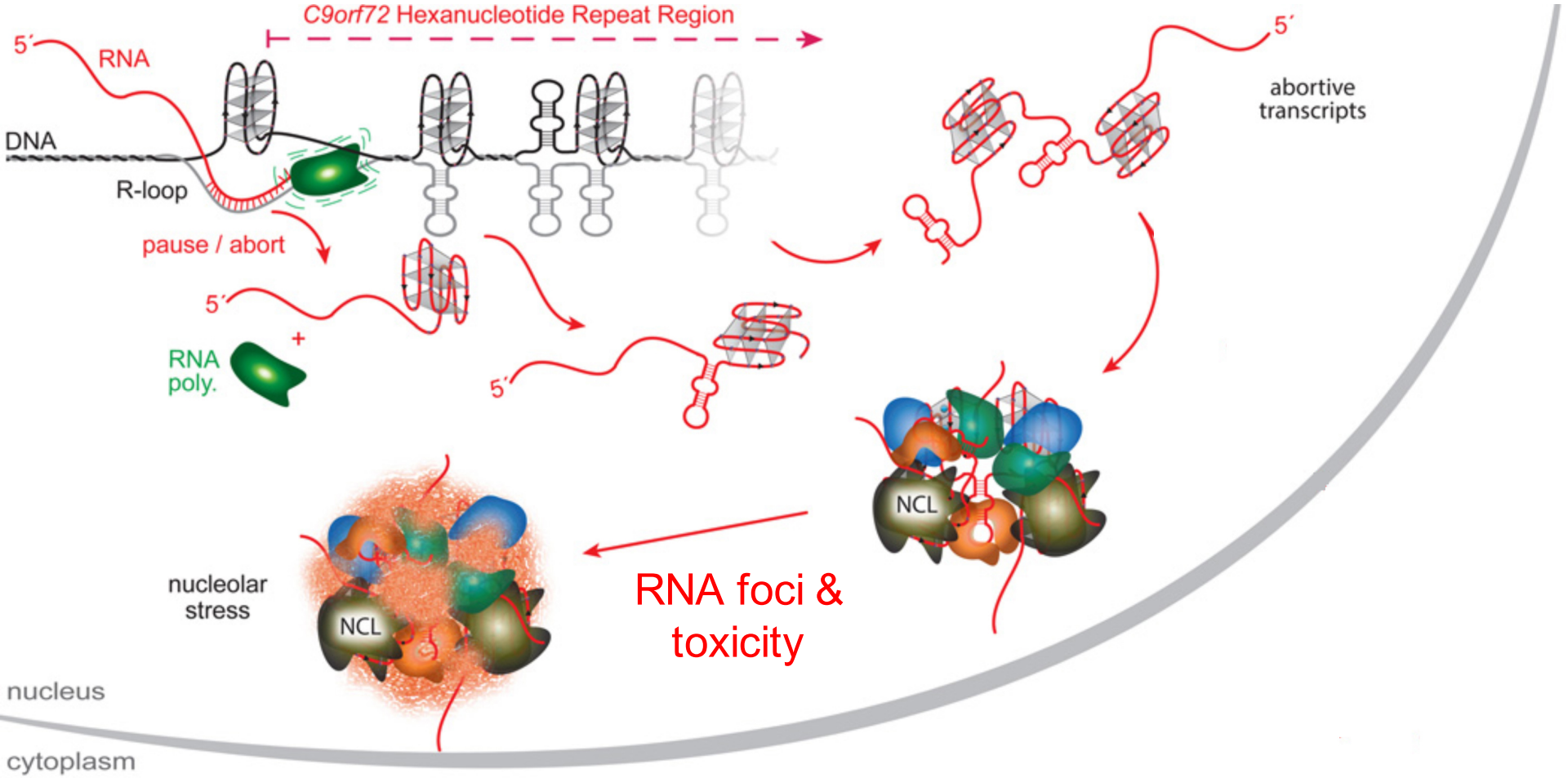
FISH probe: CY3-labelled $(GGCCCC)_4$

Potential RNA-binding proteins:
- Serine/arginine-rich splicing factor 1 (SRSF1)
-hnRNP A2/B1

RNA *in situ* hybridization (FISH) in paraffin-embedded sections from FTLD-TDP patients

DeJesus-Hernandez et al., *Neuron* 2011

A model for the molecular cascade resulting from C9ORF72 hexanucleotide expansion



Haeusler et al., *Nature* 2014

Lee et al., *Cell Reports* 2013

The *C9orf72* GGGGCC Repeat Is Translated into Aggregating Dipeptide-Repeat Proteins in FTLD/ALS

Kohji Mori,[1,*] Shih-Ming Weng,[2,*] Thomas Arzberger,[3] Stephanie May,[2] Kristin Rentzsch,[2] Elisabeth Kremmer,[4] Bettina Schmid,[2,5] Hans A. Kretzschmar,[3] Marc Cruts,[6,7] Christine Van Broeckhoven,[6,7] Christian Haass,[1,2,5] Dieter Edbauer[1,2,5,†]

Science 339, 1335-1338 (2013)

Neuron
Report

Neuron 77, 639-646 (2013)

Unconventional Translation of *C9ORF72* GGGGCC Expansion Generates Insoluble Polypeptides Specific to c9FTD/ALS

Peter E.A. Ash,[1,3,4] Kevin F. Bieniek,[1,3,4] Tania F. Gendron,[1] Thomas Caulfield,[1] Wen-Lang Lin,[1] Mariely DeJesus-Hernandez,[1,3] Marka M. van Blitterswijk,[1] Karen Jansen-West,[1] Joseph W. Paul III,[1] Rosa Rademakers,[1] Kevin B. Boylan,[2] Dennis W. Dickson,[1] and Leonard Petrucelli[1,*]
[1]Department of Neuroscience
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Mayo Clinic Florida, Jacksonville, FL 32224, USA
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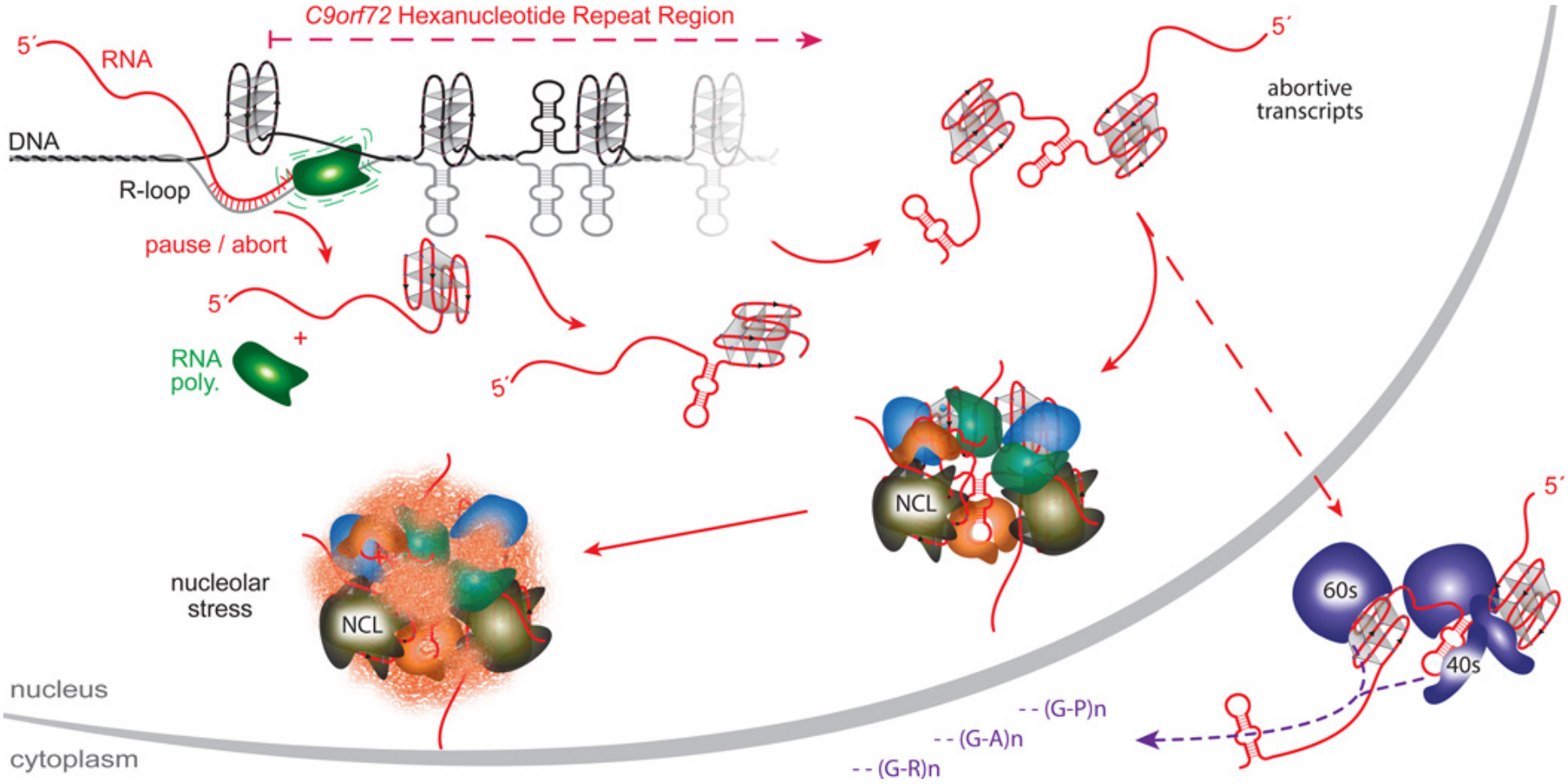
*Correspondence: petrucelli.leonard@mayo.edu
http://dx.doi.org/10.1016/j.neuron.2013.02.004

A model for the molecular cascade resulting from C9ORF72 hexanucleotide expansion

Premature transcription



RAN translation

Haeusler et al., *Nature* 2014

Lee et al., *Cell Reports* 2013

repeat associated non-ATG (RAN) translation

- Unconventional mode of translation that occurs in the absence of an initiating ATG codon.

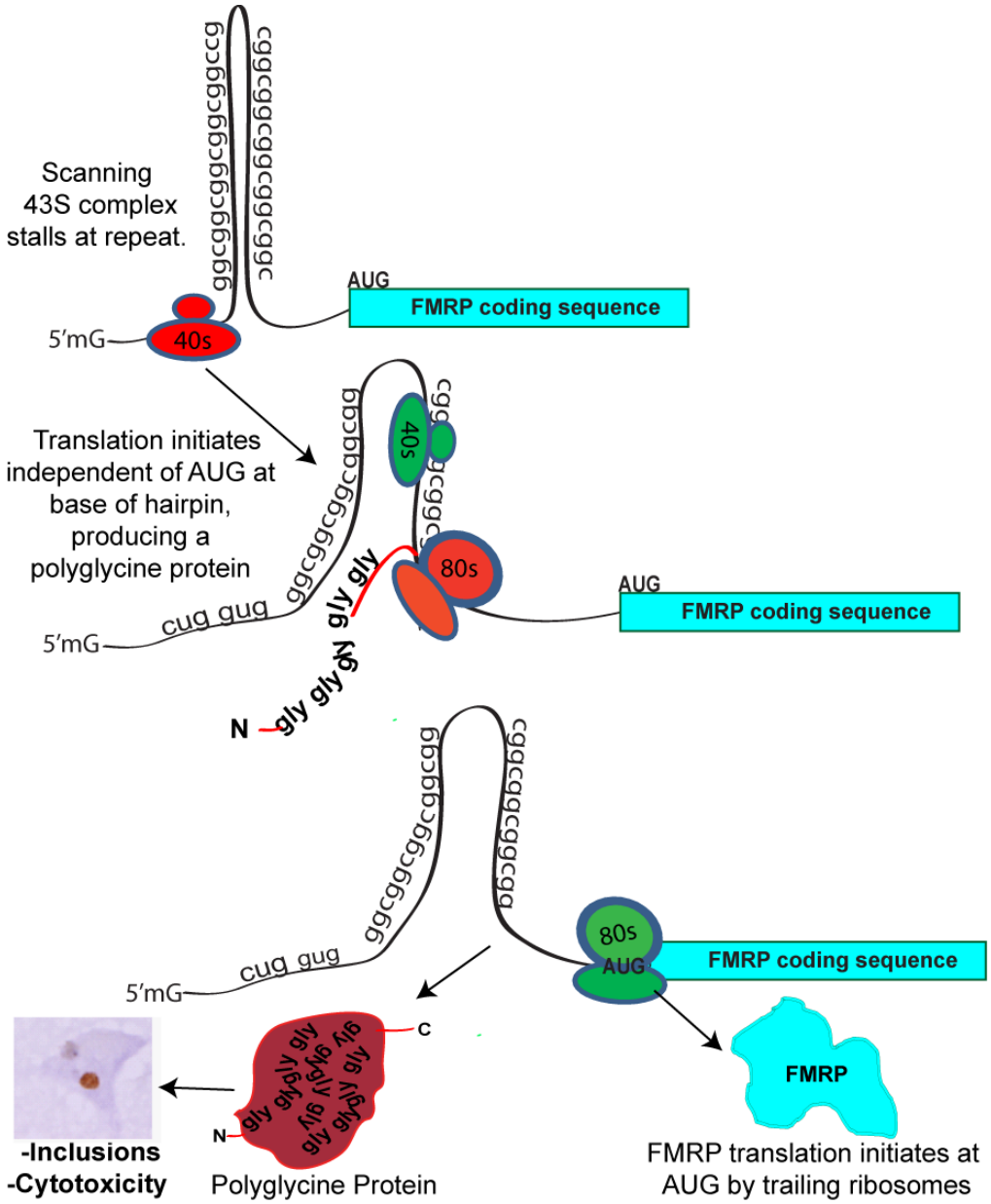
- First reported that RAN translation occurs in all reading frames (CAG, AGC, GCA) across expanded CAG repeats and produces homopolymeric proteins of long polyglutamine, polyserine, or polyalanine tracts.

  > polyalanine proteins were found in SCA8.

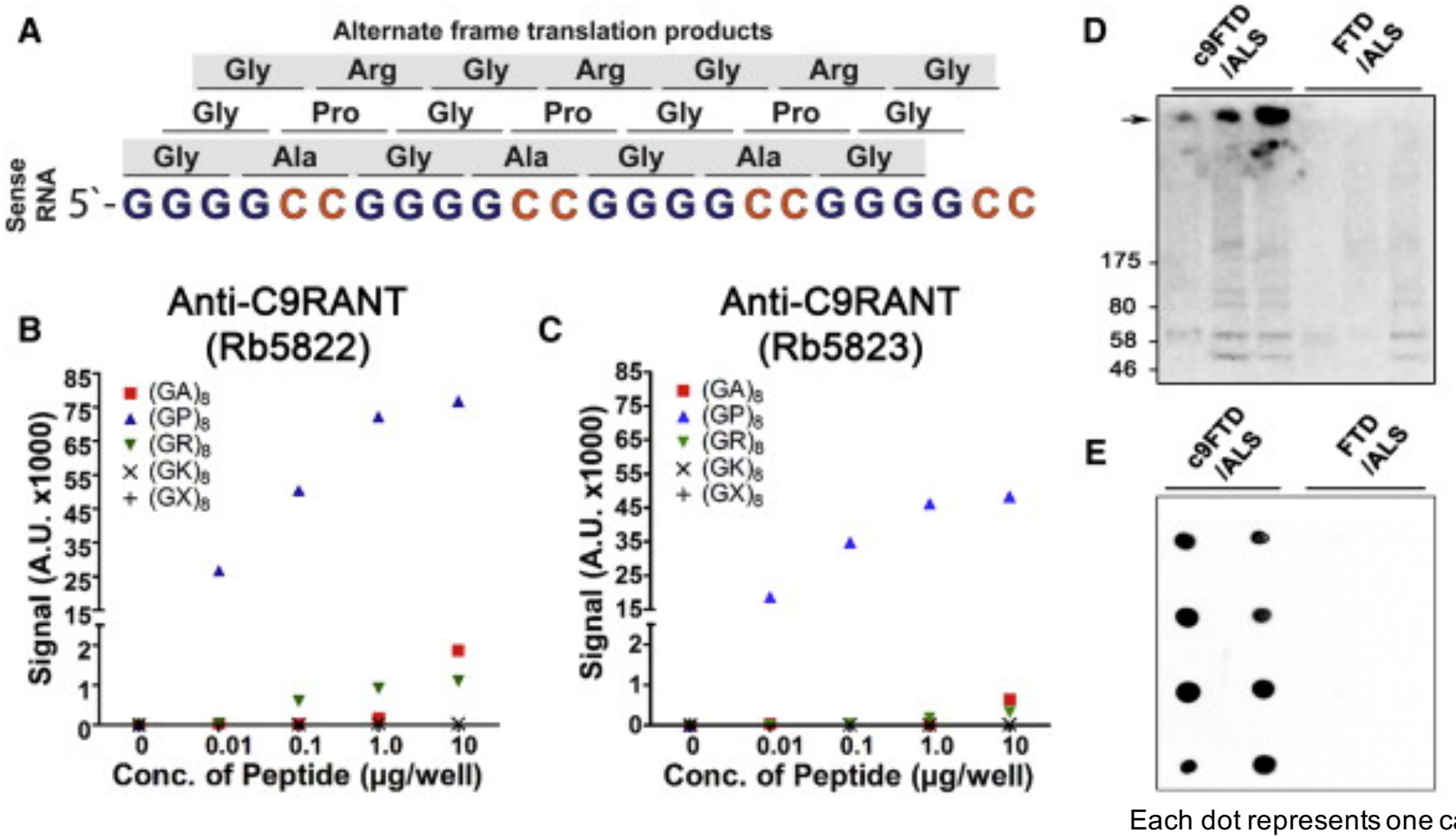
  > polyglutamine proteins were found in DM1.

- RAN translation of expanded CAG repeats depends on hairpin formation.
  (C:G complementary pairing)

Mode for CGG RAN translation in FXTAS



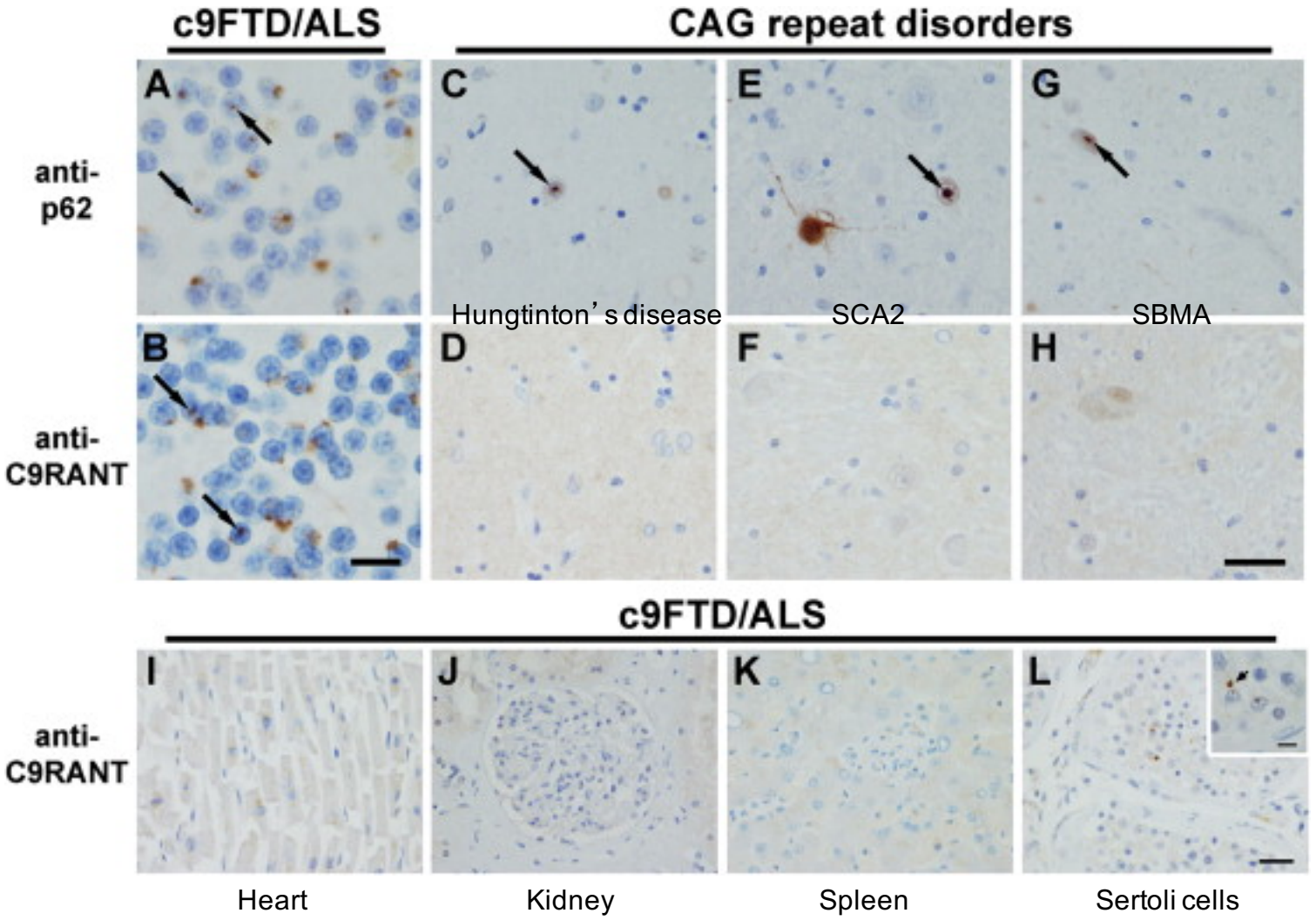
Immunoreactivity of anti-C9ORF72 in c9FTD/ALS



Each dot represents one case

Ash et al., *Neuron* 2013

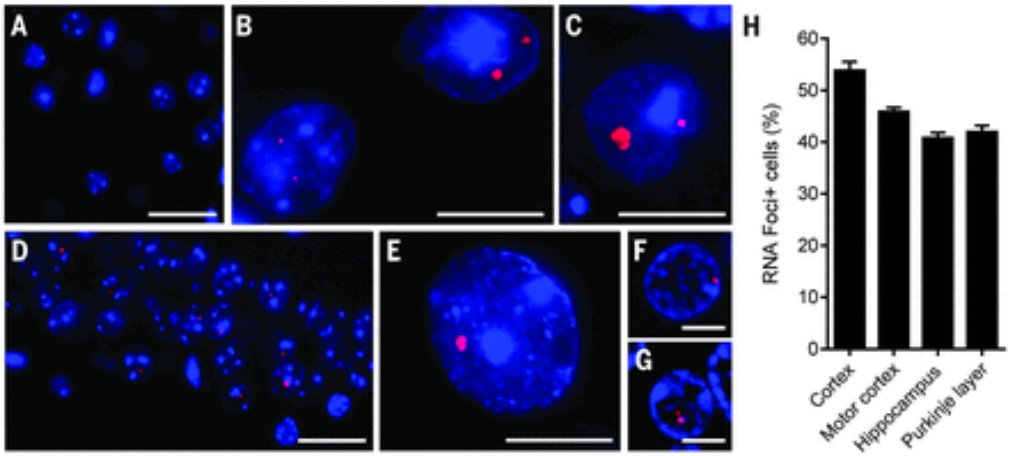
C9RANT-immunoreactive inclusions are specific to c9FTD/ALS



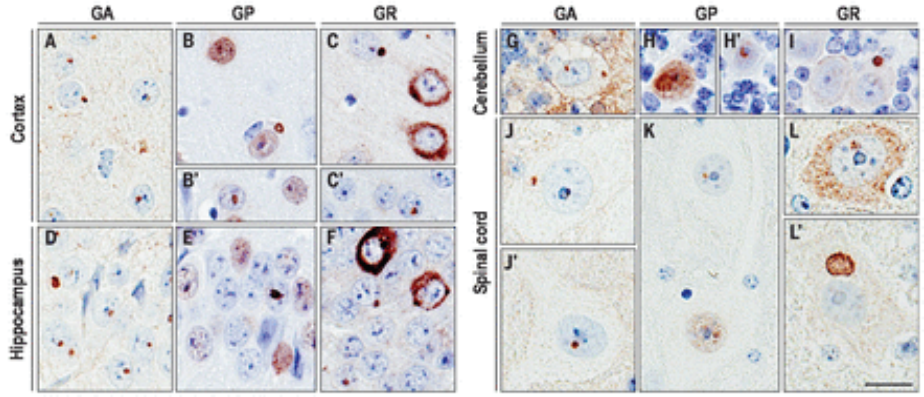
Ash et al., *Neuron* 2013

C9ORF72 repeat expansions in mice cause neuronal loss and behavioral deficits

- AAV2/9-(G4C2)2 & AAV2/9-(G4C2)66, lacking an ATG start codon
  - Intranuclear RNA foci detected in the CNS of (G4C2)66 mice



  - c9RNA protein pathology detected in the CNS of (G4C2)66 mice



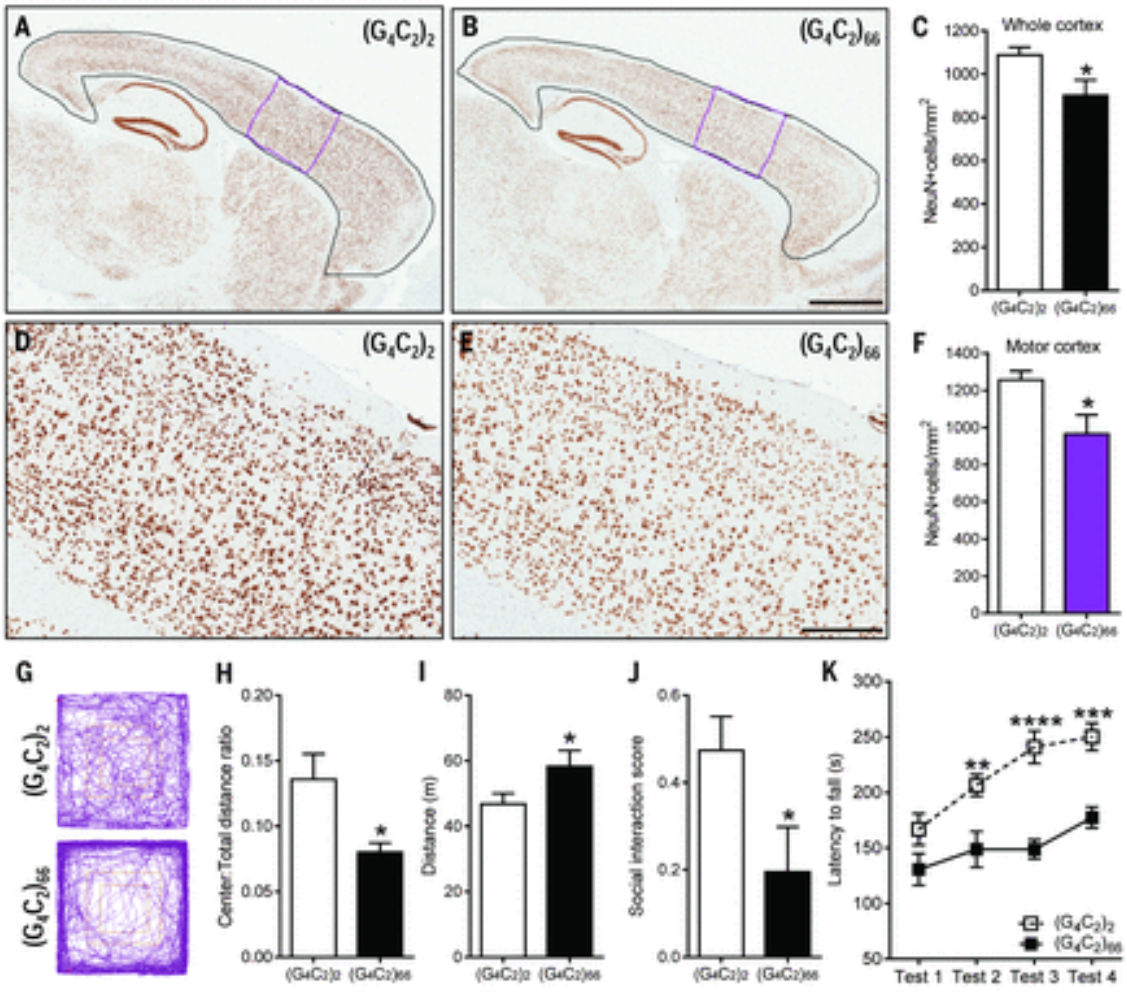
Chew et al., *Science* 2015

- AAV2/9-(

- Intra

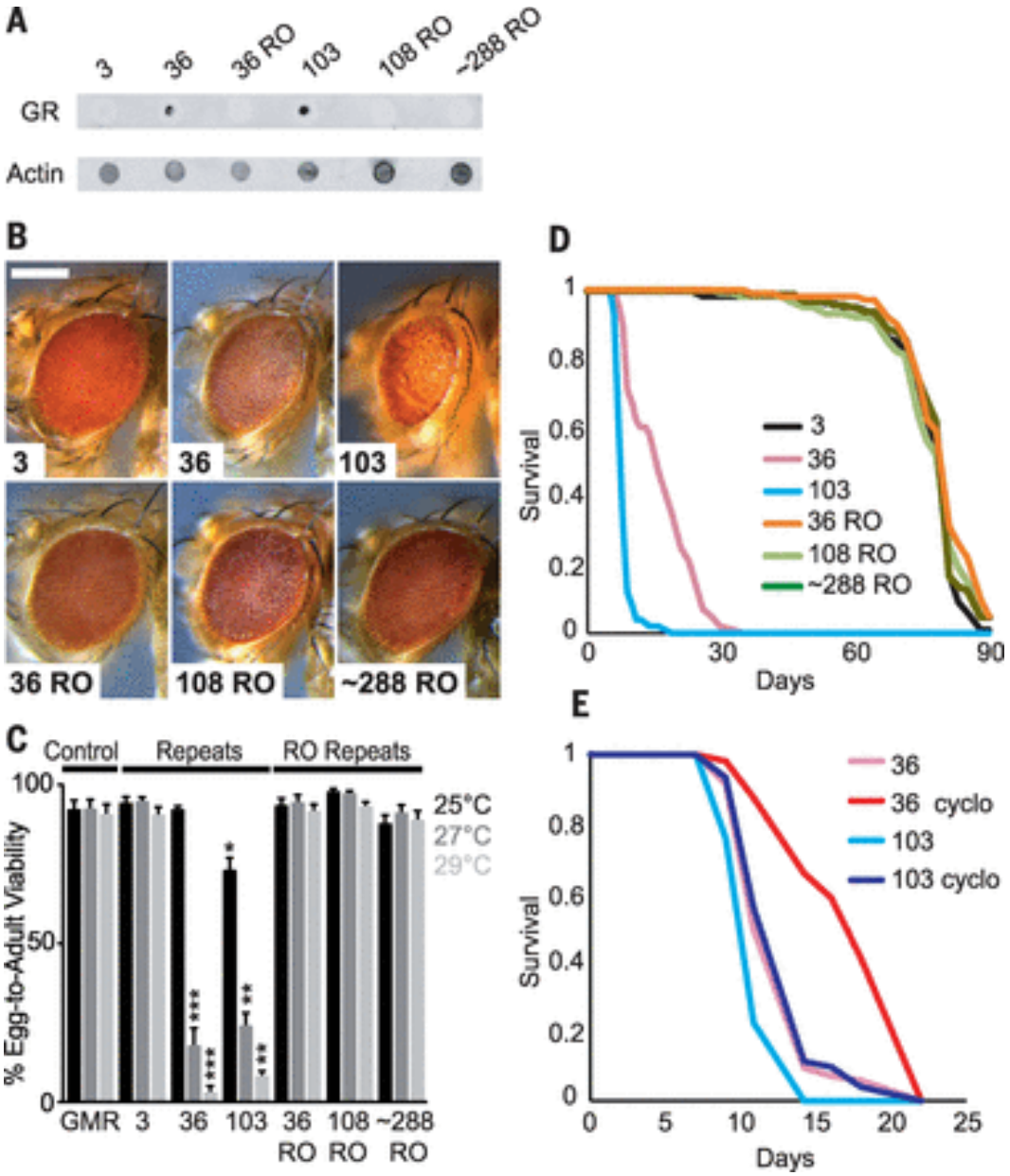
- c9R

- Cortical neurn loss & behavioral and motor deficits



Chew et al., *Science* 2015

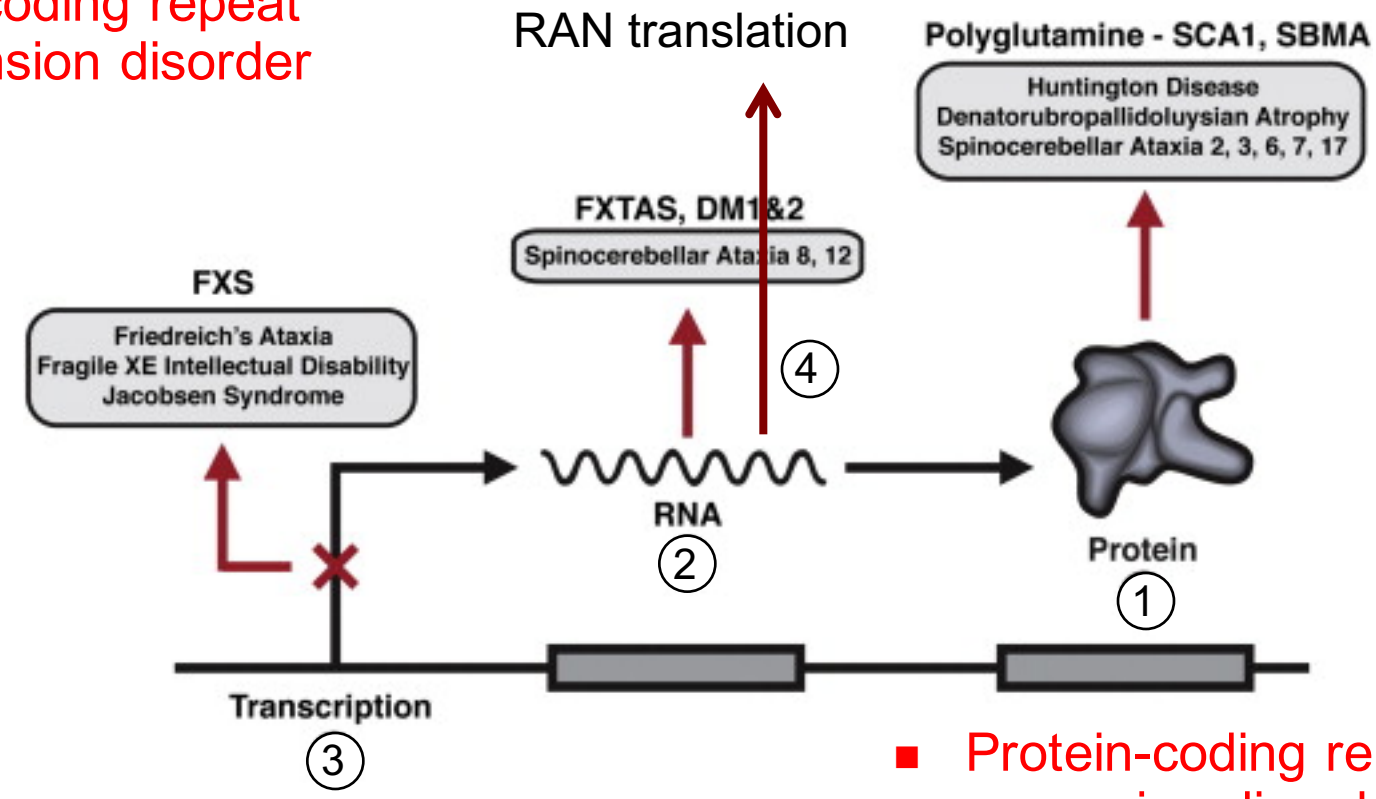
Pure GGGGCC repeats causes toxicity via dipeptide repeat proteins



Zhang et al., *Nature* 2015
Freibaum et al., *Nature* 2015
Mizielinska et al., *Science* 2014

"Four" pathogenic mechanisms for nucleotide repeat expansion disorders

- Non-coding repeat expansion disorder



- Protein-coding repeat expansion disorder

1. Protein gain-of-function
2. Loss of gene function
3. RNA gain-of-function
4. RAN protein gain-of-function

Modified from Nelson et al., *Neuron* 2013

Neuron

RAN Translation in Huntington Disease

Highlights

- RAN translation occurs across canonical ORF
- Sense and antisense RAN proteins accumulate in Huntington brains
- HD-RAN proteins are toxic to cells
- HD-RAN protein accumulation and aggregation is CAG length dependent

Authors

Monica Bañez-Coronel, Fatma Ayhan, Alex D. Tarabochia, ..., Anthony T. Yachnis, Juan C. Troncoso, Laura P.W. Ranum

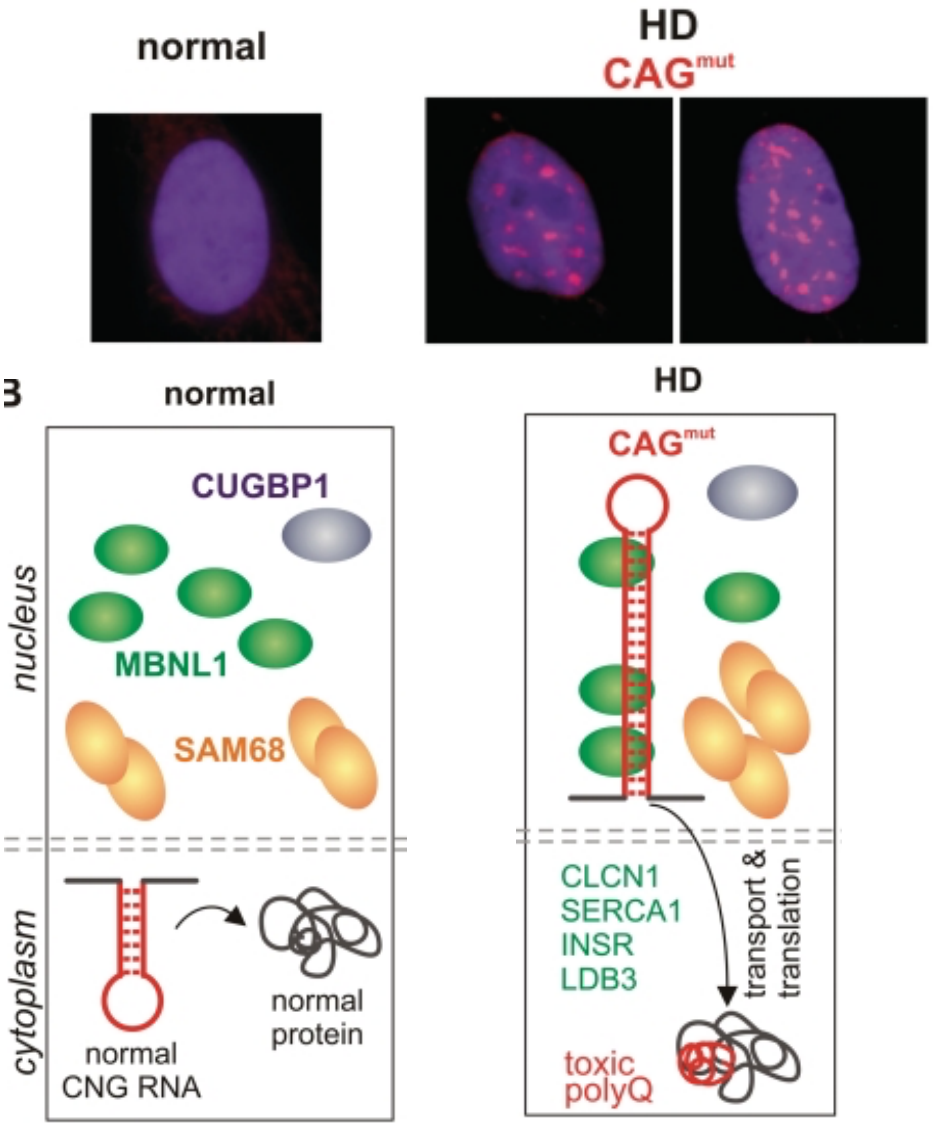
Correspondence

ranum@ufl.edu

In Brief

Bañez-Coronel et al. show the CAG*CTG expansion mutation that causes Huntington disease (HD) produces novel expansion proteins (polyAla, polySer, polyLeu, and polyCys). These repeat-associated non-ATG (RAN) proteins are expressed in a length-dependent manner and accumulate in brain regions most affected in HD.

In HD cells, expanded CAG repeat RNA forms RNA foci and partially sequesters MBNL1

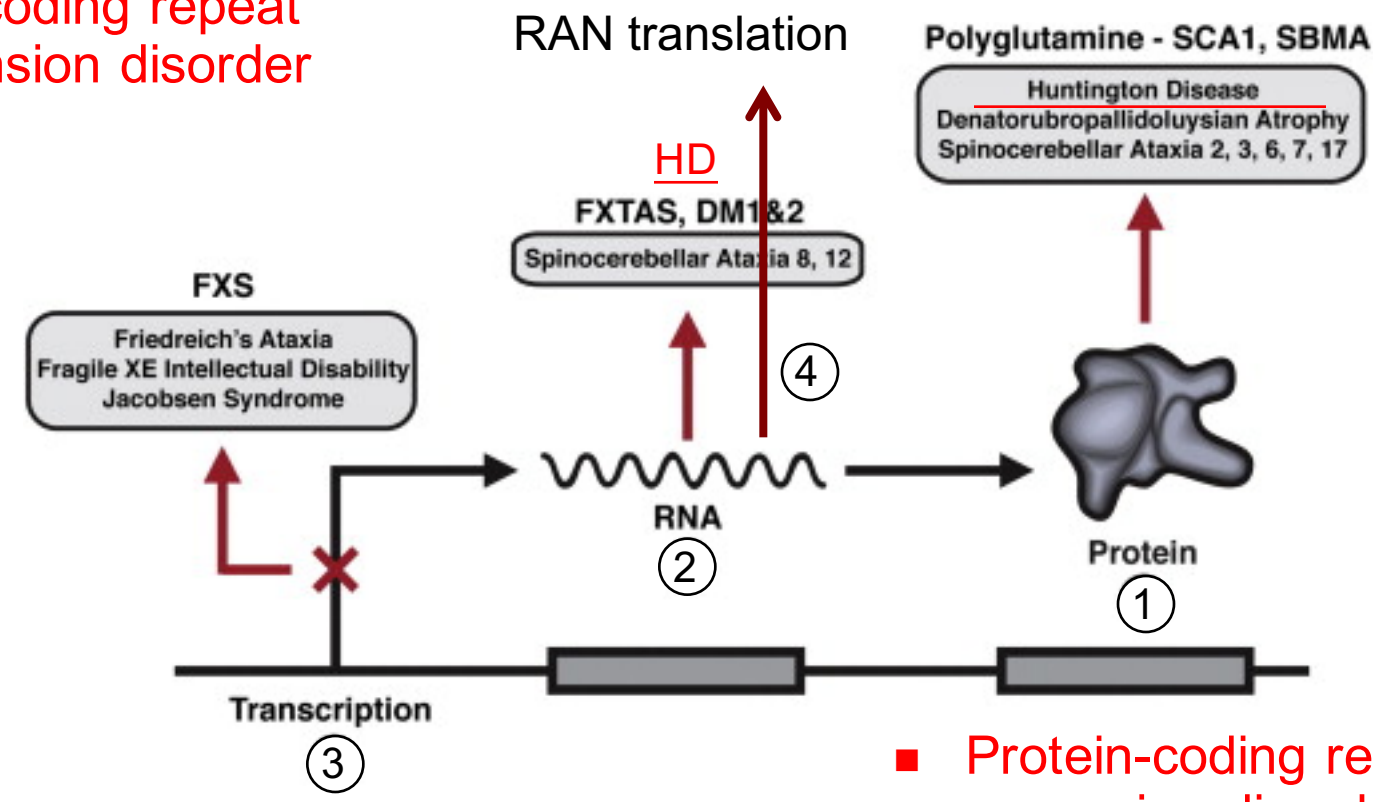


Nuclear RNA foci

Krzyzosiak et al., *Nucleic Acids Res.* 2011

"Four" pathogenic mechanisms for nucleotide repeat expansion disorders

- Non-coding repeat expansion disorder



- Protein-coding repeat expansion disorder

1. Protein gain-of-function
2. Loss of gene function
3. RNA gain-of-function ???
4. RAN protein gain-of-function ???

Modified from Nelson et al., *Neuron* 2013

Lecture Overview

- Alzheimer's disease
- Nucleotide expansion (neurodegenerative) disorders and their key pathogenic mechanisms
- Relatively new disease pathogenesis
  - spreading of mutant disease-causing aggregates

Transcellular spreading of huntingtin aggregates in the *Drosophila* brain

Daniel T. Babcock[1] and Barry Ganetzky[1]

Laboratory of Genetics, University of Wisconsin, Madison, WI 53706

Contributed by Barry Ganetzky, August 14, 2015 (sent for review May 12, 2015; reviewed by Leo J. Pallanck)

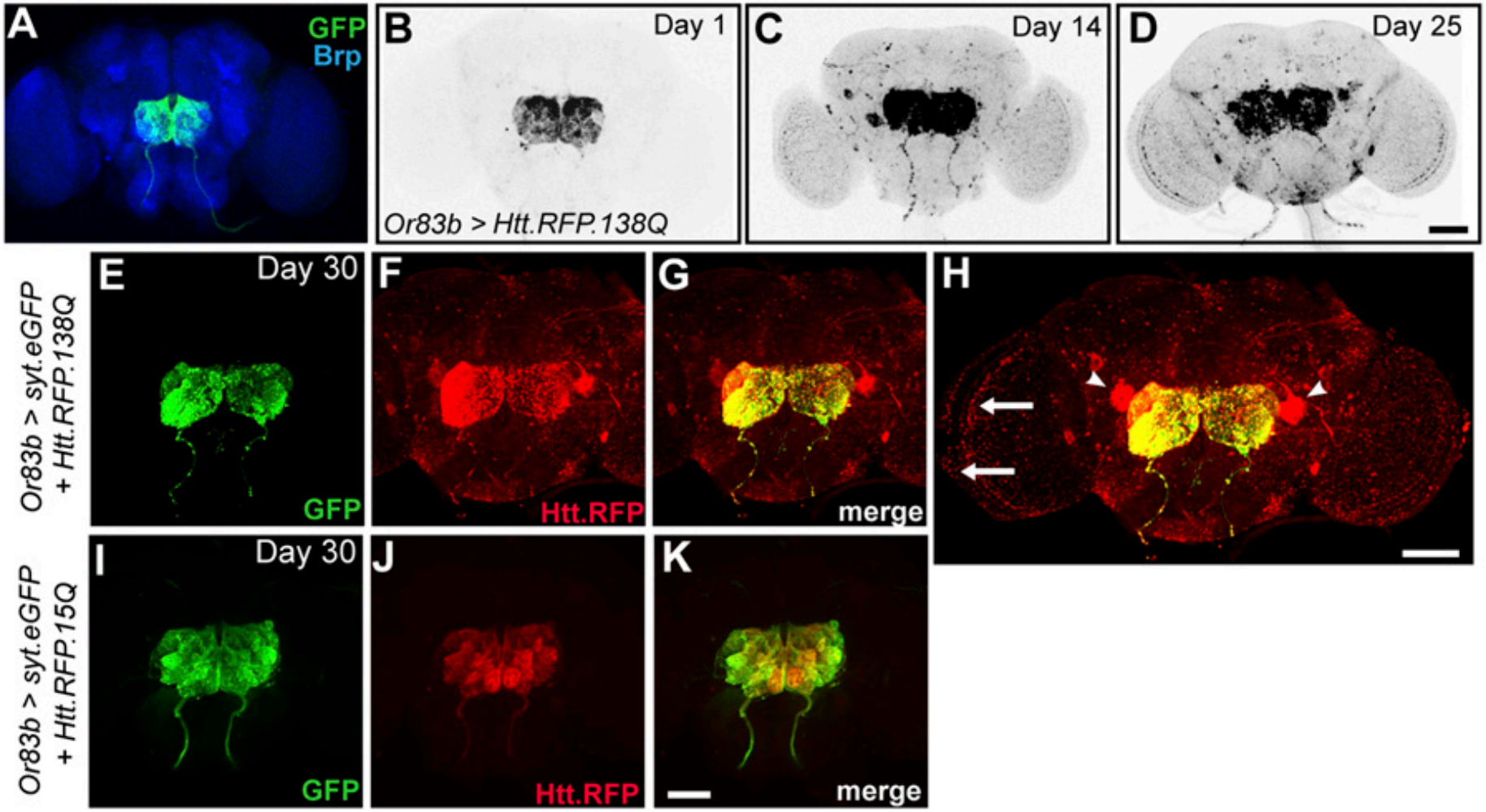
A key feature of many neurodegenerative diseases is the accumulation and subsequent aggregation of misfolded proteins. Recent studies have highlighted the transcellular propagation of protein aggregates in several major neurodegenerative diseases, although the precise mechanisms underlying this spreading and how it relates to disease pathology remain unclear. Here we use a polyglutamine-expanded form of human huntingtin (Htt) with a fluorescent tag to monitor the spreading of aggregates in the *Drosophila* brain in a model of Huntington's disease. Upon expression of this construct in a defined subset of neurons, we demonstrate that protein aggregates accumulate at synaptic terminals and progressively spread throughout the brain. These aggregates are internalized and accumulate within other neurons. We show that Htt aggregates cause non–cell-autonomous pathology, including loss of vulnerable neurons that can be prevented by inhibiting endocytosis in these neurons. Finally we show that the release of aggregates requires *N*-ethylmalemide–sensitive fusion protein 1, demonstrating that active release and uptake of Htt aggregates are important elements of spreading and disease progression.

Huntington's disease | neurodegeneration | transmission | disease model | expanded triplet repeat

manipulate separate populations of neurons simultaneously by using the yeast Gal4/Upstream Activating Sequence (UAS) (20) and bacterial LexA/LexA operator (LexAop) (21) binary expression systems. Additionally, the ability to rapidly identify and characterize genetic and chemical modifiers of this spreading phenomenon should help unravel mechanisms responsible for spreading.

In this study, we demonstrate that mutant huntingtin aggregates accumulate at synaptic terminals in the antennal lobe of the *Drosophila* central brain when expressed in olfactory receptor neurons (ORNs). Over time, these aggregates begin to spread to various regions of the brain, where they are internalized by other populations of neurons, resulting in some instances in loss of these neurons. This neuronal loss is prevented by blocking endocytosis, suggesting that spreading requires active internalization of the pathogenic protein. We observe unique spreading patterns when huntingtin is expressed in different populations of neurons, supporting the idea that nearby cells and neuronal circuits are likely targets of spreading. However, rapid accumulation of aggregates far from the original source also suggests that transmission is not limited to these circuits. The release of aggregates depends on *N*-ethylmaleimide–sensitive fusion protein 1 (NSF1), suggesting that soluble NSF attachment protein receptor (SNARE)-mediated fusion events are required for aggregate

Htt aggregates spread throughout the *Drosophila* brain



Babcock and Ganetzky, *PNAS* 2015

Potential cellular pathways involved in spreading of pathogenic proteins

- Synaptic connections
- Transcellular spreading of huntingtin aggregates in the *Drosophila* eye (Babcock and Ganetzky, *PNAS* 2015)
- Transneuronal propagation of mutant huntingtin contributes to non-cell autonomous pathology in neurons (Pecho-Vrieseling et al., *Nat. Neurosci.* 2014)

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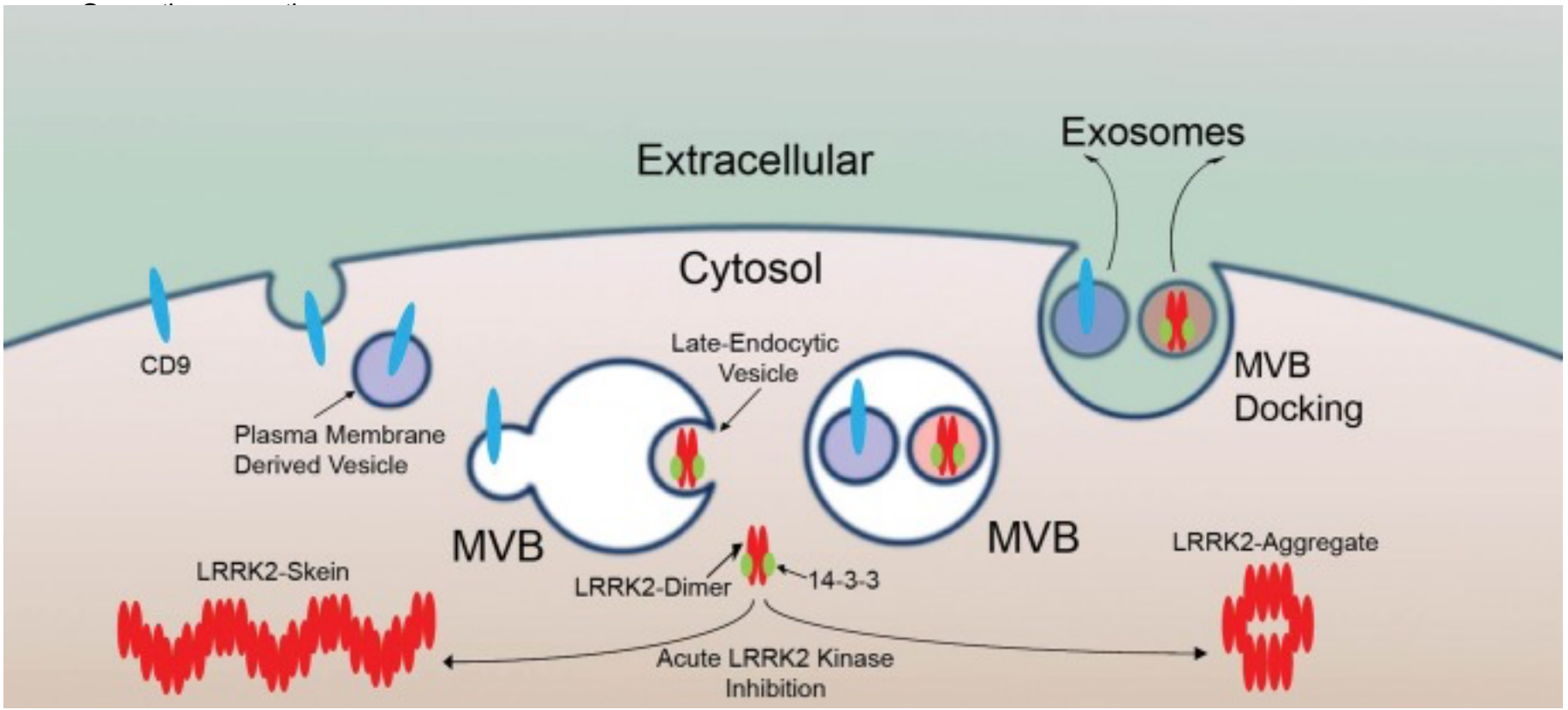
- Exosomes (a.k.a. extracellular microvesicles)
  - Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein (Desplats et al., *PNAS* 2009)
  - LRRK2 secretion in exosomes is requlated by 14-3-3 (Fraser et al., *Hum. Mol. Genet.* 2013)

Potential cellular pathways involved in spreading of pathogenic proteins



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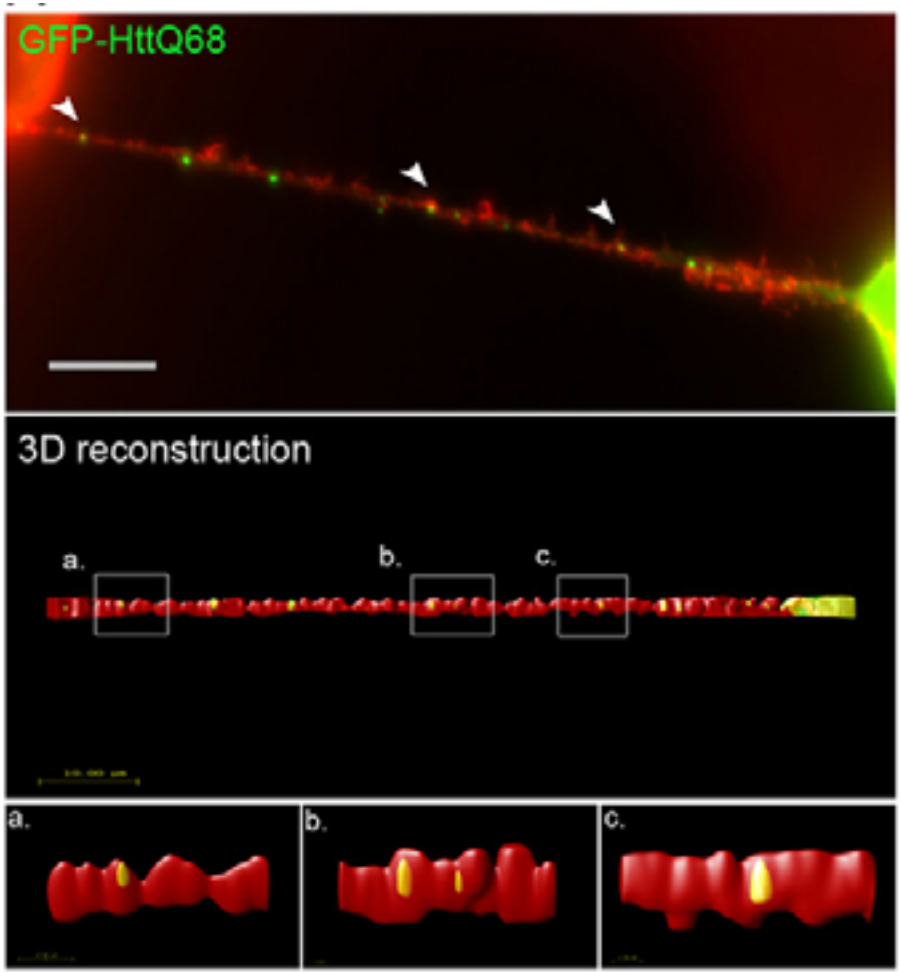
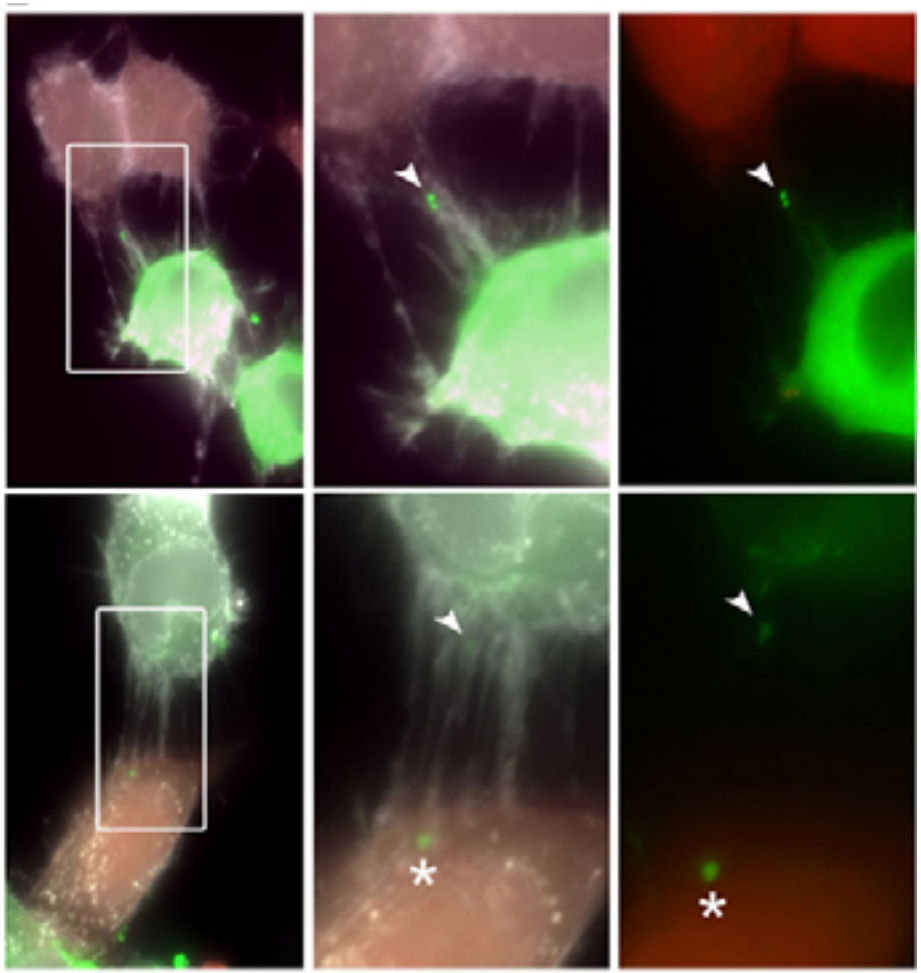
- Nanotubes

- Transfer of polyglutamine aggregates in neuronal cells occurs in tunneling nanotubes (Costanzo et al., *J. Cell Sci.* 2013)

Transfer of GFP-Htt480-68Q aggregates occurs through tunneling nanotubes in co-cultured CAD cells

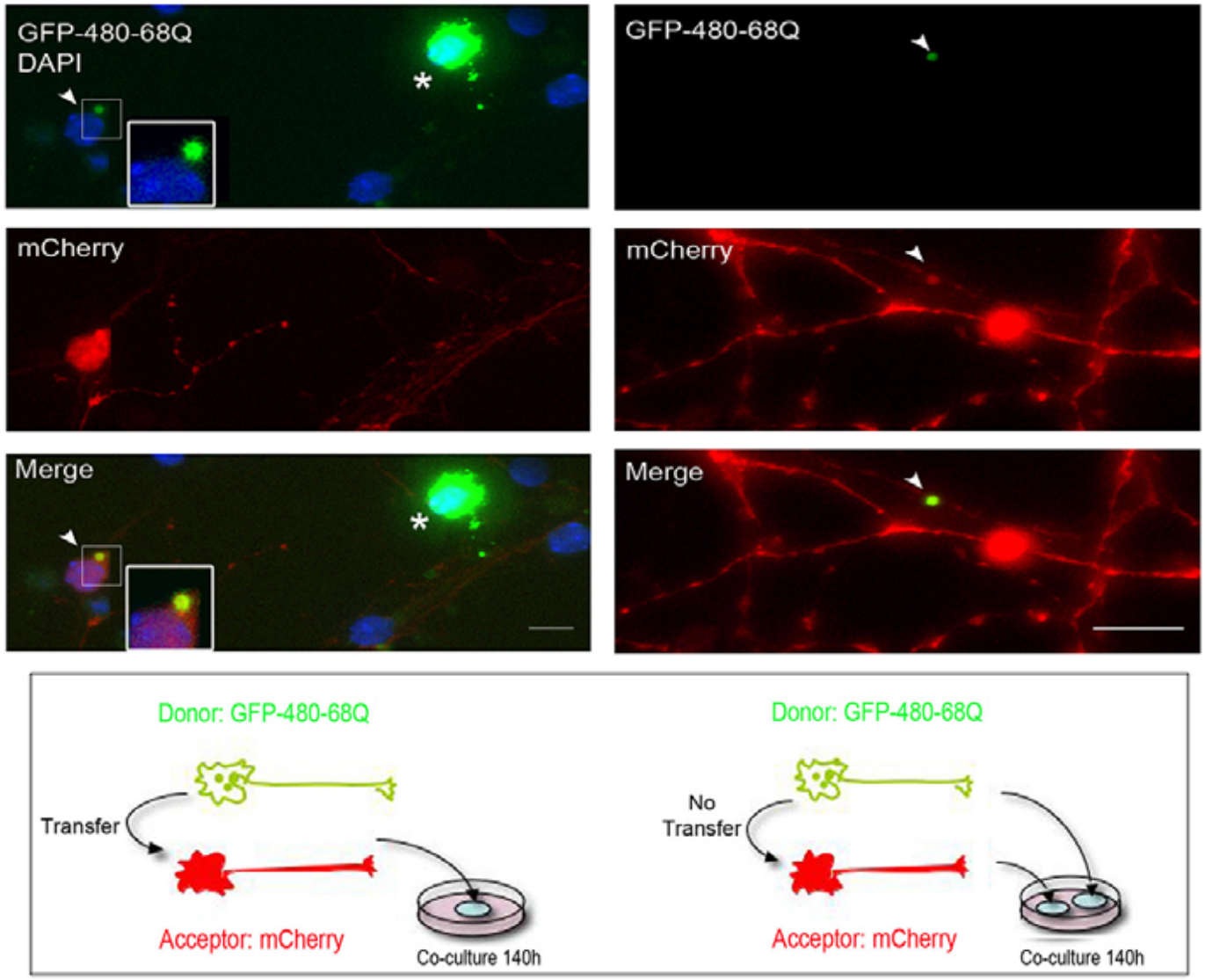




CAD cell line: Mouse (B6/D2 F1 hybrid) catecholaminergic neuronal tumor

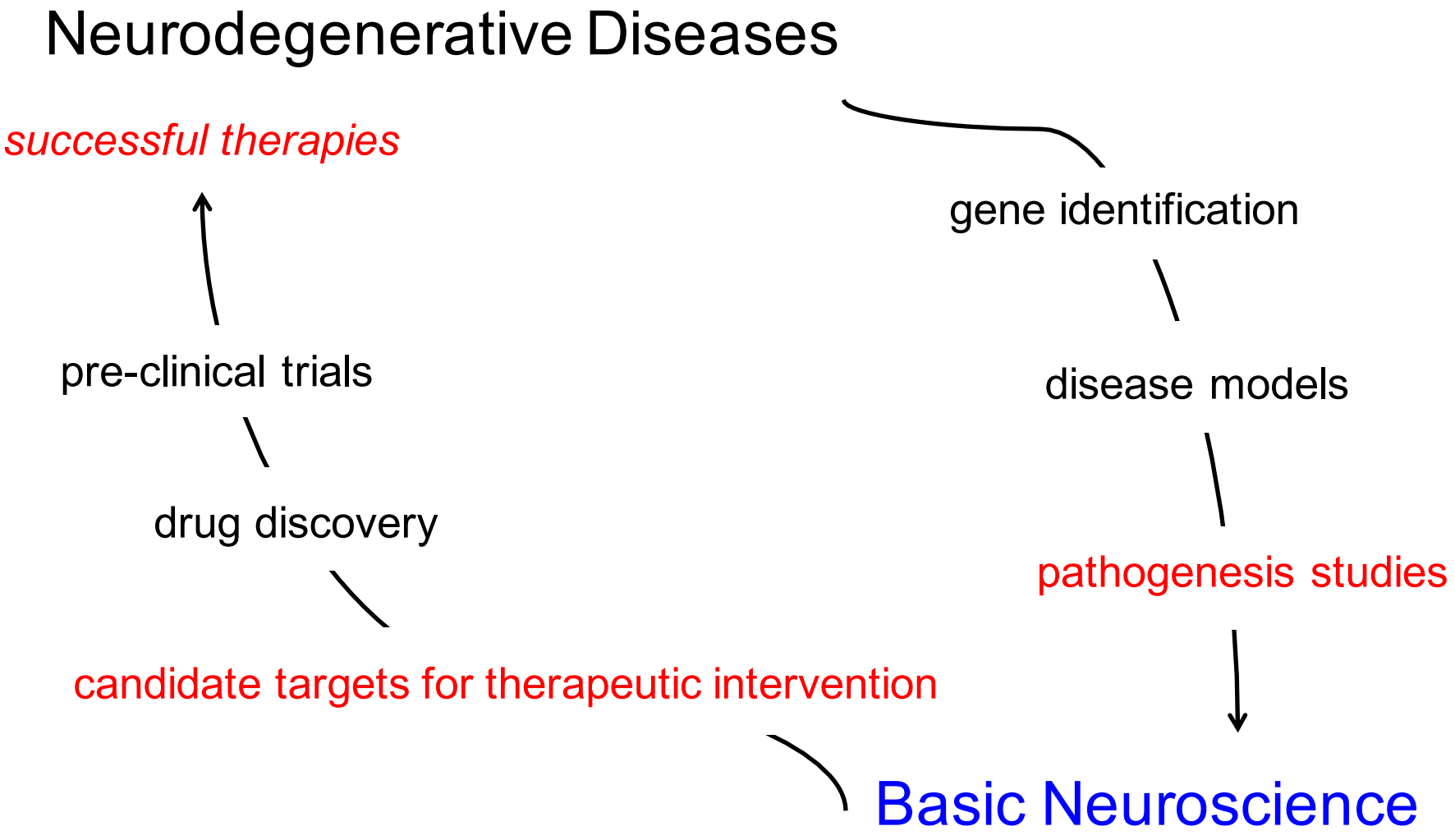
Costanzo et al., *J. Cell Sci.* 2013

GFP-Htt480-68Q aggregates transfer between primary cerebellar granular neuron co-cultures



Costanzo et al., *J. Cell Sci.* 2013

Translational Neuroscience



Neuron
Article

Neuron 80, 415-428 (2013)

RNA Toxicity from the ALS/FTD *C9ORF72* Expansion Is Mitigated by Antisense Intervention

Christopher J. Donnelly,[1,5] Ping-Wu Zhang,[1,5] Jacqueline T. Pham,[3] Aaron R. Haeusler,[4] Nipun A. Mistry,[1,5] Svetlana Vidensky,[1,5] Elizabeth L. Daley,[1,5] Erin M. Poth,[2] Benjamin Hoover,[1,5] Daniel M. Fines,[1,5] Nicholas Maragakis,[1] Pentti J. Tienari,[6] Leonard Petrucelli,[7] Bryan J. Traynor,[1,8] Jiou Wang,[2,4] Frank Rigo,[9] C. Frank Bennett,[9] Seth Blackshaw,[2] Rita Sattler,[1,5,10,*] and Jeffrey D. Rothstein[1,2,3,5,10,*]

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[8]Laboratory of Neurogenetics, National Institute on Aging, National Institute of Health, 35 Convent Drive, Room 1A-1000, Bethesda, MD 20892, USA
[9]Isis Pharmaceuticals, 2855 Gazelle Court, Carlsbad, CA 92010, USA
[10]These authors contributed equally to this work
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http://dx.doi.org/10.1016/j.neuron.2013.10.015

Neuron
Article

Sustained Therapeutic Reversal of Huntington's Disease by Transient Repression of Huntingtin Synthesis

Neuron 74, 1031-1044 (2012)

Holly B. Kordasiewicz,[1] Lisa M. Stanek,[2] Edward V. Wancewicz,[3] Curt Mazur,[3] Melissa M. McAlonis,[1] Kimberly A. Pytel,[1] Jonathan W. Artates,[1] Andreas Weiss,[4] Seng H. Cheng,[2] Lamya S. Shihabuddin,[2] Gene Hung,[3] C. Frank Bennett,[3] and Don W. Cleveland[1,*]

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*Correspondence: dcleveland@ucsd.edu
DOI 10.1016/j.neuron.2012.05.009

SUMMARY

The primary cause of Huntington's disease (HD) is expression of huntingtin with a polyglutamine expansion. Despite an absence of consensus on the mechanism(s) of toxicity, diminishing the synthesis of mutant huntingtin will abate toxicity if delivered to the key affected cells. With antisense oligonucleotides (ASOs) that catalyze RNase H-mediated degradation of huntingtin mRNA, we demonstrate that

huntingtin, i.e., at downstream targets in one of the many potential pathways possibly involved in HD pathogenesis (Melone et al., 2005).

Irrespective of the many mechanistically divergent proposals for the underlying toxicity of expanded huntingtin, a therapy aimed at diminishing the synthesis of the toxic mutant protein is an approach that will directly target the primary disease mechanism(s), as long as it is effective in the key HD-affected cells and any coincident suppression of wild-type huntingtin is tolerated. Gene silencing strategies that suppress the synthesis of huntingtin that could be deployed as potential therapeutics include

Translational Neuroscience